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TOXIC SUBSTANCES

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MEMORANDUM

Subject: **GAMMA CYHALOTHRIN:** Review of Toxicity and Mutagenicity Studies Submitted to Support Petition For Section 3 Product Registration.

PC 128807

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I. CONCLUSIONS

The Science Information Management Branch (SIMB) has evaluated the new subchronic and the developmental toxicity studies in rats as well as the battery of mutagenicity/genetic toxicity studies for gamma cyhalothrin (technical and formulated products). The Data Evaluation Records are attached to this Memorandum. All of the studies are classified as Acceptable/Guideline. The overall toxicity database for gamma cyhalothrin is adequate for a Section 3 Registration. This conclusion is based on "bridging" the toxicity studies conducted with the other two isomers, cyhalothrin and lambda cyhalothrin shown in Tables 1-3. No additional toxicity studies are required at this time for the registration of gamma cyhalothrin.

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II. ACTION REQUESTED

Pytech Chemicals GMBH, has submitted a petition for Section 3 Product Registration of gamma cyhalothrin for non-food/feed use. Gamma cyhalothrin is a resolved isomer of the pyrethroid insecticide cyhalothrin, and as such shares physical, chemical, and biological properties with both cyhalothrin (PC Code: 128867) and lambda cyhalothrin (PC Code: 128897). Gamma cyhalothrin is the most active isomer of cyhalothrin/lambda cyhalothrin, and thus the technical gamma cyhalothrin product may be considered a refined form of cyhalothrin/lambda cyhalothrin that has been purified by removal of less active and inactive isomers. The following studies were submitted in support of registration: Subchronic Toxicity Study- Rat (MRID No.45447321 and 45447322), Prenatal Developmental Toxicity Study - Rat (MRID 45447323, 45447324), and Mutagenicity Studies (MRID Nos.: 45447401, 45447402, 45447403, 45447404, 45447405, 45447325, 45447326, 45447327).

III. GAMMA CYHALOTHRIN STUDIES REVIEWED

Subchronic Toxicity Study- Rat MRID No.45447321 and 45447322

Executive Summary In a 28 day range finding study (MRIDs 45447322) gamma cyhalothrin was administered to 10 Wistar Han rats/sex/group in the diet at dose levels of 0, 2.5, 10, 50, 125, or 250 ppm (0/0, 0.2/0.2, 0.8/1.0, 4.2/4.5, 8.8/10.2, and 8.2/9.5 mg/kg/day in males/females) for 4 weeks. At 125 ppm, decreased body weight, body weight gain and food consumption was observed. Clinical signs of postural and muscular abnormalities, clear perioral soiling, in coordination, decreased extensor-thrust and touch response, and increased salivation were observed. Males showed increased levels of alanine and aspartate aminotransferase (27% and 20%, respectively), urea nitrogen (14%) and relative kidney weights (11%). In females, total protein and albumin were decreased (12-14%), and relative kidney weight was increased (6%). Increased levels of protein and ketones were found in the urine of both sexes. The 250 ppm, group was terminated on Day 5 due to moribundity.

In a subchronic toxicity study (MRIDs 45447322), XR-225 (gamma cyhalothrin; 100% a.i) was administered to 10 Wistar Han rats/sex/group in the diet at dose levels of 0, 2.5, 10, 50, or 100 ppm (equivalent to 0/0, 0.2/0.2, 0.7/0.8, 3.4/4.2, and 6.6/8.0 mg/kg/day in males/females) for 13 weeks. Additionally, 10 Wistar rats/sex/group were similarly treated at 0 and 100 ppm for 13 weeks and were observed for additional 4 weeks of recovery period. No treatment-related effects were observed at 2.5, 10, or 50 ppm. Moreover, no adverse effects were observed on, food consumption, ophthalmology, hematology, or mixed function oxidase activity. Minor decreases in body weight and body weight gains were observed at 100 ppm but were not considered treatment related. At 100 ppm, 3/20 males died due to the treatment. At 100 ppm, animals showed clinical signs of incoordination (2 males and 3 females vs 0 controls) and decreased extensor-thrust (3 each sex treated vs 0 controls). At 100 ppm, dermatitis was observed in the females from Days 64-91 (3-4 treated vs 0 controls). In females (n= 9-10) at Week 13, the incidence of alopecia and skin ulcer increased (20-30% treated vs 0% controls), as did the

incidence of skin acanthosis, hair follicle atrophy, and ulcer (20% treated [each lesion] vs 0% controls). In males at Week 13, increase in relative kidney weights (19%), and increased incidence of slight kidney tubular degeneration (67% treated vs 10% controls) were observed. However, these kidney effects were minimal and not dose dependent. Other microscopic lesions were increased in incidence (n = 9-10) and were considered to be equivocally treatment-related as follows: (i) thyroid cysts in males (89% treated vs 40% controls); (ii) very slight to slight lung perivascular inflammation in females (50% treated vs 10% controls); and (iii) very slight to slight mesenteric tissue inflammation in females (40% treated vs 0% controls).

In the recovery group (100 ppm), no treatment-related observations in both sexes,. except for one female exhibiting dermatitis at Day 119. Alopecia and skin ulcer were observed in 10% treated (each lesion) vs 0% controls. Recovery in the animals was also evident during pathology. In blood serum, significant increase in urea nitrogen (22%), decrease in albumin and glucose (14% and 21%, respectively) were observed in the 100 ppm females at Week 13. Glucose was decreased ($p \leq 0.05$) by 17% during recovery in the 100 ppm group females at Week 17.

The LOAEL was 100 ppm (6.6/8.0 mg/kg/day in males/females), based on mortality in males, neuromuscular effects in both sexes; dermatitis, and gross and microscopic skin lesions in females. The NOAEL is 50 ppm (3.4/4.2 mg/kg/day in males/females).

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements (OPPTS 870.3100a) for a Subchronic oral toxicity study in the rat.

Prenatal Developmental Toxicity Study - Rat MRID 45447323, 45447324

Executive Summary: In the range finding study (MRID 45447323), gamma cyhalothrin (100.0% a.i) was given at the levels of 1, 2.5, 5, and 15 mg/kg/day to 8 pregnant female CD rats/group. Excessive maternal toxicity was observed including severe neurological symptoms at doses ≥ 5 mg/kg body weight. At 1.0 mg/kg bw, animals showed decreased body weights by 22% generally through out treatment. At 2.5 mg/kg, incoordinated gait (2/8 dams), clear perioral stain (7/8 animals), and clear or red periocular stain (1/8 animals) were observed in animals. Significant decrease in body weight (7%) on GD 21 and in body weight gain (19-35%) was observed on GD 15-21, GD 6-21, and GD 0-21. Terminal body weights were significantly decreased (7%). In this range finding study, the maternal NOAEL was not observed and LOAEL was 1.0 mg/kg/day, based on decreased body weight gains. No other effects of treatment were noted. The developmental LOAEL was not observed and NOAEL was 2.5 mg/kg/day. The dose levels for the main study (discussed below) were based on the results of this range finding study.

In the main developmental toxicity study (MRID 45447324), XR-225, gamma cyhalothrin; (100.0%) in corn oil was administered daily by oral gavage at a dose volume of 4 mL/kg bw to 25 pregnant female CD rats/group at dose levels of 0, 0.1, 0.5, or 2.0 mg/kg/day on gestation days (GD) 6 through 20. All dams were sacrificed on GD 21; their fetuses were removed by cesarean and examined.

At 2.0 mg/kg, one animal exhibited multiple signs of toxicity on GD 21, including decreased activity, red vulvar discharge, red perioral staining, and red body soiling. Clear perioral soiling was observed in 16/25 dams on multiple occasions. Decreased feces (3/25 dams) with abdominal urine soiling (1/25) and partially closed eye lid (1/25) were noted in animals. Significant treatment related effects were observed in maternal body weights and food consumption. Body weight was decreased by 7% on GD 21; body weight gains were decreased by 16-53% on GDs 6-9, 18-21, throughout treatment when not corrected for gravid uterine weights (GD 6-21) and when corrected for gravid uterine weights (statistics not performed), and for the overall (GD 0-21) study. Gravid uterine weights were comparable to controls. Terminal body weights were decreased by 7% in the 2.0 mg/kg dams. Food consumption was decreased by 12-20% in the 2.0 mg/kg dams on GD 6-12 and 18-21.

The maternal LOAEL is 2.0 mg/kg/day, based on decreased body weights, body weight gains, and food consumption, and clinical signs of toxicity (e.g., clear perioral staining). The maternal NOAEL is 0.5 mg/kg/day.

No effects of treatment were noted on numbers of litters, live fetuses, early resorptions, or on sex ratio or post-implantation losses. There were no abortions, premature deliveries, complete litter resorptions, or dead fetuses. Late resorptions were observed at 2.0 mg/kg (12 treated vs 0 controls); however, 11 of these occurred in one litter. There were no treatment-related alterations in fetal growth. There were no treatment-related external, visceral, or skeletal malformations or variations. **The developmental toxicity NOAEL is 2.0 mg/kg/day; a LOAEL was not established.**

This study is classified **Acceptable/Guideline)** and satisfies the requirements (OPPTS 870.3700a for a developmental study in the rat.

Mutagenicity Studies

MRID Nos.: 45447401, 45447402, 45447403, 45447404, 45447405, 45447325, 45447326, 45447327

i. Gene Mutations

Executive Summary: In independent repeat mammalian cell forward gene mutation assays (MRID 45447404), cultures of mouse lymphoma L5178Y cells, heterozygous at the thymidine kinase locus ($TK^{+/-}$), were exposed for four hours to XR-225 (100% a.i., dissolved in dimethyl sulfoxide, DMSO), in the presence and absence of exogenous metabolic activation provided by hepatic microsomes from Aroclor 1254-treated rats (± S9), at concentrations ranging from 3.9 to 1000 µg/mL +S9, and from 156 to 4500 µg/mL -S9. In addition to cultures exposed to the solvent (DMSO), other cultures were treated with the mutagens, methyl methanesulfonate (MMS) and 20-methylcholanthrene, the latter chemicals to serve as positive controls for the non-activated and activated test series. At harvest, forward mutation ($TK^{-/-}$) was assessed by counting the numbers of colonies resistant to the analog of the nucleic acid base, pyrimidine, 6-trifluoropyrimidine (6-TFT), which permits only mutants to survive.

In preliminary cytotoxicity tests, moderate to severe decreases in relative suspension growth (RSG) were evident in non-activation cultures treated at ≥ 70.3 µg/mL ± S9, but beginning at lower concentrations, ≥ 12.5 µg/mL, in activated cultures. Addition of test material also resulted in cloudiness of the treatment medium at ≥ 70.3 µg/mL, with increasing precipitation at ≥ 1125 µg/mL. In the main mutagenicity assays, excessive toxicity (decreasing plating efficiencies) was evident in activated cultures exposed to ≥125-250 µg/mL, but only moderate toxicity was found in non-activated cultures exposed to higher doses (2000-4500 µg/mL). Precipitation was evident at ≥ 1000 µg/mL.

At no dose in any assay, ± S9, however, did XR-225-treated cultures manifest increased frequencies in mutant colonies over concurrent negative (solvent) controls, or background control data. In contrast, both positive controls displayed marked increases in mutant colonies. **Therefore, XR-225 is considered non-mutagenic in this mouse lymphoma forward mutation assay at the thymidine kinase locus.**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements (OPPTS 870.5100) for and *in vitro* mammalian gene mutation data.

Executive Summary: In independently repeat (initial and confirmatory) reverse gene mutation assays in bacteria (MRID 45447325), cultures of four histidine-deficient (*his⁻*) strains of *Salmonella typhimurium* (TA98,, TA100, TA1535, and TA1537) and the tryptophan-deficient (*try⁻*) WP2 *uvr*A strain of *Escherichia coli* were exposed by the preincubation modification of the standard Ames procedure to XR-225 (100% a.i., in dimethyl sulfoxide, DMSO). Following incubation for 52 ± 4 hours at 37 ± 2°C at six concentrations ranging from 10 to 5000 µg/plate, revertants to histidine and tryptophan prototrophy (*his⁺*, *try⁻*) were scored and compared to the incidences in concurrent control (DMSO) cultures. Additional cultures were treated with strain-specific mutagens, to serve as positive controls.

In the range-finding test, cytotoxicity (as evidenced by thinning of background lawn) was observed in non-activated (-S9) TA-100 cultures at ≥ 1000 µg/plate, but not at any dose up to the limit, 5000 µg/plate, in either S9-activated TA100 or WP2 *uvr*A⁻. Slight precipitation of the test material was also found with both tester strains at ≥ 333 µg/plate, progressing to moderate at 3330 and 5000 µg/plate ± S9.

In the mutagenicity assays, however, no increase in test plate revertants over controls were observed in any strain at any dose, in the presence or absence of S9- mix activation. All positive controls responded with marked increases in revertants.

Therefore, XR-225 (supercyhalothrin) is considered non-mutagenic in the standard battery of bacterial strains.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements (OPPTS 870.5100) for an *in vitro* mutagenicity (bacterial reverse gene mutation) data.

Executive Summary: In independent repeat (initial and confirmatory) pre-incubation reverse gene mutation assays in bacteria (MRID 45447327), cultures of four histidine-deficient (*his*$^-$) strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and the trytophan-deficient (try$^-$) WP2 *uvr*A strain of *Escherichia coli* were exposed to GF-231 (a formulation containing 14.7% XR-225 dissolved in water), at concentrations up to the limit dose, 5000 µg/plate, in the presence and absence of exogenous metabolic activation consisting of hepatic S9-homogenates from Aroclor-1254 treated male rats. In addition to concurrent vehicle (H_2O) controls, other cultures were treated with strain-specific mutagens. Following plate incubation at 37 ± 2° C for 52 ± 4 hours, the frequencies of revertants to prototrophy (*his*$^+$, *try*$^+$) in test cultures were compared to vehicle control values.

In preliminary dose range-finding tests (with TA100 and WP2 *uvr*A), cytotoxicity (reduced background lawn) was evident at ≥ 333 µg/plate in non-activated GF-231- treated TA100 cultures. Slight precipitation of test substance was observed in both species ± S9 at the two highest doses, 3333 and 5000 µg/plate.

In the main mutagenicity assays, cytotoxicity and precipitation at the same dose levels were observed in test article-treated cultures, but at no concentration up to the cytotoxic-precipitating limit dose were statistically increased frequencies of revertants to histidine or tryptophan prototrophy found in either test substance-treated strain, in the presence or absence of metabolic activation. By contrast, all positive controls exhibited marked increases in revertants. **Therefore, the formulation GF-231 (14.7% XR-225 a.i.) is considered non-mutagenic in this battery of bacterial strains.**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements (OPPTS 870.5100) for an *in vitro* mutagenicity (bacterial reverse gene mutation) data.

Executive Summary: In independent repeat (initial and confirmatory) preincubation assays in bacteria (MRID 45447402), cultures of four histidine-deficient (*his*$^-$) strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and the tryptophan-deficient (*try*$^-$) strain of *Escherichia coli* WP2 *uvr*A were exposed to GF-317 (a formulation containing 6 % XR-225 a.i., dissolved in water, H_2O) at six concentrations ranging from 33.3 to 5000 µg/plate, in the presence and absence of exogenous metabolic activation (± S9) provided by hepatic microsomes from Aroclor 1254-treated rats. Additional cultures were exposed to water alone, or to strain-specific mutagens, to serve as vehicle and positive controls, respectively. Following plate incubation at 37 ± 2° C for 52 ± 4 hours, the numbers of revertant colonies (*his*$^-$, *try*$^+$) in test cultures were compared to those in vehicle controls.

No evidence of cytotoxicity (thinning or absence of the background bacterial lawn and/or reduction in revertants) was observed at any dose up to the limit, 5000 µg/plate, in any tested strain, either in the presence or absence of activation. Further, **the test compound did not induce any significant increase in revertants,** compared to marked increases induced by mutagen-treatment.
Therefore, GF-317 is considered non-mutagenic in the standard battery of bacterial strains.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements (OPPTS 870.5100) for an *in vitro* mutagenicity (bacterial reverse gene mutation) data.

ii. Cytogenetics

Executive Summary: In an *in vivo* mammalians cytogenetics (micronucleus) assay (MRID 45447326), groups of mice (each, six males and six females) were administered XR-225 (100% a.i., dissolved in 10 mL/kg corn oil) by oral gavage on two consecutive days at doses of 1, 2 and 4 mg/kg/day, and sacrificed 24 hours after the second dose. Equal numbers of mice (6M:6F) were administered two consecutive doses of the corn oil vehicle, or two consecutive doses of 120 mg/kg cyclophosphamide monohydrate, and sacrificed 24 hours after dosing, to serve as negative (vehicle) and positive controls. At sacrifice, femoral bone marrow from each animal was processed cytologically and the frequencies of micronucleated polychromatic erythrocytes (MNPCE) from XR-225-treated mice were compared to vehicle control values.

In prior dose range-finding tests (4 mice/sex) at: (i) twice 10, 50, or 200 mg/kg/day; or (ii) twice 5 mg/kg/day; or (iii) twice 0.5, 2.5, or 5 mg/kg/day), severe signs of toxicity (tonoclonic convulsions, uncoordinated gait, hind limb splay, etc.) were observed at levels above 2.5 mg/kg/day, and deaths at ≥ 5 mg/kg/day.

In the main micronucleus assay, dose-related adverse clinical effects (perineal soiling, and/or increased "reactivity", occasional uncoordinated gait, convulsions) were observed at the mid (2 mg/kg/day) and high (4 mg/kg/day) dosages, but no effect on erythropoiesis (% PCE).

There were no statistically significant increases in the frequencies of MNPCEs in test article-treated mice compared to those in negative control animals. The positive control animals manifested statistically significant decreases in percentage of PCE, and statistically significant increases in MNPCEs.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements (OPPTS 870.5395) for an *in vivo* cytogenetic mutagenicity data.

Executive Summary: In a cytogenetics (micronucleus) assay (MRID 45447401), groups of male mice (6/dose/group) were administered GF-231 (14.7% a.i., dissolved in distilled water, DW) by oral gavage on two consecutive days at doses of 50, 100 and 200 mg/kg/day, and sacrificed after the second dose. An additional group of six males was administered the highest dose, HDT (200 mg/kg/day), to replace animals that may die at this dose. Additional groups of six males were given consecutive doses of DW, or a single dose of the mutagen, cyclophosphamide (CP, 120 mg/kg), and sacrificed 24 hours after their last dose, to serve as vehicle (negative) and positive controls, respectively. At sacrifice, femoral bone marrow was examined for the presence of micronucleated polychromatic erythrocytes (MNPCEs); the frequencies of MNPCEs from treated animals were compared to vehicle controls.

In a preliminary range-finding test with both sexes, treatment-related toxicity (including death, incoordiantion, increased reactivity, convulsions and perioral soiling) was observed in test article-treated animals at the HDT (200 mg/kg). Evidence of comparable animal toxicity (but no mortality) was also found in the main mutagenicity assay in animals at the HDT of GF-231, but **no significant differences from vehicle controls in erythropoieses (% PCE) or MNPCE frequencies.** In contrast, the positive control group (CP) a manifested a marked induced increase in MNPCEs accompanied by a significant decrease in % PCE.

This study is classified as **Acceptable/Guideline**, and satisfies the guideline requirements (OPPTS 870.5395) for an *in vivo* cytogenetic mutagenicity data.

Executive Summary: In a cytogenetics assay (MRID 45447403), groups of male mice (6/dose) were administered GF-317 (a formulation containing 5.9% technical XR-225 a.i. dissolved in distilled water, DW) by oral gavage at two consecutive daily doses of 300, 600 and 1000 mg/kg/day, and sacrificed 24 hours after the second treatment. Additional groups of six males were given two consecutive doses of the vehicle (DW) alone, or a single dose of the mutagen, cyclophosphamide monohydrate (CP, 120 mg/kg), and sacrificed 24 hours after the last dose, to serve as negative and positive controls, respectively. At sacrifice, femoral bone marrow from each animal was processed for the evaluation of micronucleated polychromatic erythrocytes (MNPCEs). The frequencies of MNPCEs in bone marrow from treated animals were compared to DW controls.

In preliminary range-finding tests, most mice (both sexes treated) administered consecutive oral doses of GF-317 ranging from 100 to 1000 mg/kg/day showed major signs of moderate to severe clinical toxicity at ≥ 600 mg/kg/day, including one death (female) given 1000 mg/kg/day. There was, however, no evidence of an effect on body weight or body temperature.

In the main micronucleus assay (using males only), comparable degrees of clinical toxicity were observed at ≥ 600 mg/kg/day, including the death of all six 600 mg/kg/day animals, as well as six males given 1000 mg/kg/day; all mortalities were deemed by the investigators to be treatment-related. Signs of pyrethroid toxicity was seen at 600 and 1000 mg/kg/day. No effects on body weights of test animals were found, and no significant differences in frequencies of MNPCEs between GF-317-treated groups and DW controls. By contrast, positive (CP) controls induced a marked increase in MNPCEs, and a significant decrease in % PCE. Therefore, GF-317 is considered negative for cytotoxic activity (structural chromosome aberrations).

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements (OPPTS 870.5375) for an *in vivo* cytogenetic mutagenicity data.

Executive Summary: In independent, repeat (initial and confirmatory) assays (MRID 45447405), primary lymphocyte cultures from male rats were exposed for four hours to XR-225

(100% a.i. dissolved in dimethyl sulfoxide, DMSO), in the presence and absence of hepatic homogenates (± S9 microsomes) derived from Aroclor 1254-treated male rats, to seven concentrations ranging from 4.5 to 4500 µg/mL, and harvested 20 hours later (Initial Assay-1). Based upon mitotic indices (MI), cultures treated at 45, 150 and 450 µg/ml -S9 and 45, 450 and 1500 µg/mL +S9 were selected for the determination of structural (breaks and exchanges) and numerical (polyploid) chromosomal aberrations. In a confirmatory trial (Assay-2), non-activated (-S9) cultures were exposed continuously for 24 hours to nine concentrations of XR-225 ranging from 1.0 to 500 µg/mL, or at a slightly narrower range of 12 concentrations from 1.25 to 200 µg/mL in a repeat of Assay -2, whereas activated (+S9) cultures were treated for 4 hours at six concentrations ranging from 15 to 3000 µg/mL; this series was also harvested at 24 hours. Based on reduced MIs and/or compound insolubility, non-activated cultures treated at 5, 20 and 40 µg/mL and activated cultures treated at 45, 150 and 1500 µg/mL were selected for determining chromosomal aberrations. In addition to cultures exposed to DMSO alone, others were treated with the mutagenic clastogens (chromosome-breakers) mitomycin C (MMC:0.05, 0.075, 0.5 µg/mL) and cyclophosphamide (CP:4 or 6 µg/mL), to serve as solvent (negative) controls (DMSO), and the latter as positive controls for the non-activated and activated test series, respectively.

Evidence of test substance bone marrow cytotoxicity (represented by decreases in MI) was observed in both non-activated and activated cultures, beginning at concentrations between 30 and 45 µg/mL, with dramatic decreases at ≤ 100 µg/mL, accompanied by increasing compound precipitation ≤ 450 µg/mL -S9 in Assay-1, and at 200-500 µg/mL ± S9 in the confirmatory assay. **At no concentration of X4-225 in either series of assays, however, were significant increases over concurrent solvent control or historical background values) in either structural or numerical aberrations found.** In contrast, marked and significant increases were induced in structural (but not numerical) aberrations for both positive controls.

Hence, XR-225 is considered negative for clastogenicity in this *in vitro* chromosomal aberration study in rat lymphocytes.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements (OPPTS 870.5375) for an *in vitro* cytogenetic mutagenicity data.

IV. RESULTS OF STUDIES WITH THE THREE ISOMERS

The toxicological evaluation of gamma cyhalothrin can be accomplished by "bridging" studies conducted with cyhalothrin and lambda cyhalothrin. The results of the toxicity studies with the three isomers are presented in the following Tables 1-3.

Table 1: Acute Toxicity Studies with Technical and Formulations

Pyrethroid Type	Cyhalothrin	Lambda cyhalothrin	Gamma cyhalothrin
Acute Toxicity - Technical			
Acute Oral	LD_{50} : 243 mg/kg (m) 144 mg/kg (f) **Tox.Cat. II**	LD_{50} : 79 mg/kg (m) 56 mg/kg (f) **Tox.Cat. II**	LD_{50} :> 50 mg/kg (m) 55 mg/kg (f) **Tox.Cat. II**
Acute Dermal	LD_{50} : >1000 mg/kg LD_{50} : >2000 mg/kg **Tox.Cat. II**	LD_{50} :632 mg/kg (m) 696 mg/kg(f) **Tox.Cat. II**	LD_{50} :>1500 mg/kg (m) 1643 mg/kg (f) **Tox.Cat. II**
Acute Inhalation	LC_{50} :0.173 mg/L (m) 0.183 mg/L(f) **Tox.Cat. II**	LC_{50} : 0.065 mg/L **Tox.Cat. IV**	LC_{50} : 0.042 mg/L (m) 0.028 mg/L (f) **Tox.Cat. IV**
Eye Irritation	Moderate irritant **Tox.Cat. III**	Mild irritant **Tox.Cat. II**	Moderate irritant **Tox.Cat. III**
Skin Irritation	Mild irritant **Tox.Cat. IV**	Non-irritant **Tox.Cat. IV**	Slight irritant **Tox.Cat. IV**
Dermal Sensitization	Sensitizer	Non-sensitizer	Sensitizer
Acute Toxicity - Formulations			
Formulation Type	***Grenade*** **(20% cyhalothrin)**	***Karate Z*** **(23% lambda cyhalothrin)**	***GF-231*** **(15% gamma cyhalothrin)**
Acute Oral	LD_{50} : 122 mg/kg **Tox.Cat. II**	LD_{50} : 245 mg/kg **Tox.Cat. II**	LD_{50} : 2250 mg/kg **Tox.Cat. IV**
Acute Dermal	LD_{50} : > 2000 mg/kg **Tox.Cat. IV**	LD_{50} : > 2000 mg/kg **Tox.Cat. IV**	LD_{50} :>5000 mg/kg **Tox.Cat. IV**
Acute Inhalation	LC_{50} :1.72 mg/L **Tox.Cat. IV**	LC_{50} :3.72 mg/L **Tox.Cat. IV**	LC_{50} :> 2.72 mg/L **Tox.Cat. IV**
Eye Irritation t	Not Available	Mild irritant **Tox.Cat. II**	Moderate irritant **Tox.Cat. III**
Skin Irritation	Corrosive **Tox.Cat. I**	Mild irritant **Tox.Cat. II**	Slight irritant **Tox.Cat. IV**
Dermal Sensitization	Inconclusive - excessive irritation	Mild sensitizer	Non sensitizer

Table 2: Comparative Toxicity Studies with Cyhalothrin, Lambda cyhalothrin and Gamma cyhalothrin

STUDY TYPE - ISOMER	MRID NO. CLASSIFICATION	RESULTS
ACUTE NEUROTOXICITY		
Acute Neurotoxicity - Rat **Lambda** cyhalothrin	44861510 **Acceptable/Guideline**	**NOAEL**: 10 mg/kg **LOAEL**: 35 mg/kg (clinical observations indicative of neurotoxicity and changes in FOB parameters).
Cyhalothrin	None	None
Gamma cyhalothrin	None	None
SUBCHRONIC NEUROTOXICITY		
Sub Neurotoxicity - Rat **Lambda** cyhalothrin	45689101 **Acceptable/Guideline**	**NOAEL**: 11.4 mg/kg/day **LOAEL**: Not Established
Cyhalothrin	None	None
Gamma cyhalothrin	None	None
SUBCHRONIC TOXICITY STUDIES		
28-Day Dietary - Rat **Cyhalothrin**	00153029 **Acceptable/Nonguideline**	**NOAEL**: 2 mg/kg/day **LOAEL**: 10 mg/kg/day (clinical signs of neurotoxicity). At higher doses, decreases in body weight gain and food consumption and changes in organ weights.
28-Day Dietary - Rat **Cyhalothrin**	00154806 **Acceptable/Nonguideline**	**NOAEL**: 1.0 mg/kg/day **LOAEL**: 2.0 mg/kg/day (decreases in mean body weight gain in females).
Lambda cyhalothrin	None	None
Gamma cyhalothrin	45447321 **Acceptable/Nonguideline**	**NOAEL**: male/female = 4.2/4.5 mg/kg/day **LOAEL**: male/female = 8.8/10.2 mg/kg/day. (decreased body weight, body weight gain, food consumption, clinical and biochemical effects).
13-Week Dietary -Rat **Cyhalothrin**	00154805 **Acceptable/Guideline**	**NOAEL**: 2.5 mg/kg/day **LOAEL**: 12.5 mg/kg/day (decreased body weight gain in males).

Table 2: Comparative Toxicity Studies with Cyhalothrin, Lambda cyhalothrin and Gamma cyhalothrin

STUDY TYPE - ISOMER	MRID NO. CLASSIFICATION	RESULTS
13-Week Dietary - Rat **Lambda** cyhalothrin	00153028 **Acceptable/Guideline**	**NOAEL**: 2.5 mg/kg/day **LOAEL**: 12.5 mg/kg/day (reduced body weight gain and food consumption in both sexes and food efficiency in females).
13-Week Dietary - Rat **Gamma** cyhalothrin	45447322 **Acceptable/Guideline**	**NOAEL**: male/female =3.4/4.2 mg/kg/day **LOAEL**: male/female = 6.6/8.8 mg/kg/day (mortality in males, neuromuscular effects in both sexes, dermatitis, and gross and microscopic skin lesions in females).
26-Week Dietary - Dog **Cyhalothrin**	00154795 **Acceptable/Guideline**	**NOAEL**: 1.0 mg/kg/day **LOAEL**: 2.5 mg/kg/day (increase in liquid feces. At 10.0 mg/kg/day, clinical signs of neurotoxicity).
Lambda cyhalothrin	None	None
Gamma cyhalothrin	None	None
4-Week Dietary - Mouse **Cyhalothrin**	43241901 **Acceptable/Nonguideline**	**NOAEL**: 64.2/77.9 mg/kg/day **LOAEL**: 309/294 mg/kg/day (mortality, clinical signs of toxicity, decreases in body weight gain and food consumption. changes in hematology and organ weights, minimal cetrilobular hepatocyte enlargement).
Lambda cyhalothrin	None	None
Gamma cyhalothrin	None	None
CHRONIC TOXICITY STUDY - DOG		
Chronic Toxicity - Dog **Lambda** cyhalothrin	40027902 **Acceptable/Guideline**	**NOAEL**: 0.1 mg/kg/day **LOAEL**: 0.5 mg/kg/day (clinical signs of neurotoxicity).
Cyhalothrin	None	None
Gamma cyhalothrin	None	None

Table 2: Comparative Toxicity Studies with Cyhalothrin, Lambda cyhalothrin and Gamma cyhalothrin

STUDY TYPE - ISOMER	MRID NO. CLASSIFICATION	RESULTS
CHRONIC TOXICITY/CARCINOGENICITY STUDIES- RAT & MOUSE		
Chronic Toxicity /Carcinogenicity - Rat **Cyhalothrin**	00154803 **Acceptable /Guideline**	**NOAEL**: 2.5 mg/kg/day **LOAEL**: 12.5 mg/kg/day (decreases in mean body weight) **No evidence of carcinogenicity.**
Lambda cyhalothrin	None	None
Gamma cyhalothrin	None	None
Carcinogenicity - Mouse **Cyhalothrin**	00150842 **Acceptable/Guideline**	**NOAEL**: 15 mg/kg/day **LOAEL**: 75 mg/kg/day (increased incidence of piloerection, hunched posture; decreased body weight gain in males). **No evidence of carcinogenicity.**
Lambda cyhalothrin	None	None
Gamma cyhalothrin	None	None
DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES		
Developmental Toxicity - Rat **Cyhalothrin**	00154800 **Acceptable / Guideline**	**Maternal NOAEL**: 10 mg/kg/day **Maternal LOAEL**: 15 mg/kg/day (uncoordinated limbs, reduced body weight gain and food consumption). **Developmental NOAEL**: 15 mg/kg/day **Developmental LOAEL**: Not established
Lambda cyhalothrin	None	None
Gamma cyhalothrin	45447324 **Acceptable/Guideline**	**Maternal NOAEL**: 0.5 mg/kg/day **Maternal LOAEL**: 2.0 mg/kg/day (clinical signs, reduced body weight and body weight gain and food consumption). **Developmental NOAEL**: 2.0 mg/kg/day **Developmental LOAEL**: Not established

Table 2: Comparative Toxicity Studies with Cyhalothrin, Lambda cyhalothrin and Gamma cyhalothrin

STUDY TYPE - ISOMER	MRID NO. CLASSIFICATION	RESULTS
Developmental Toxicity - Rabbit **Cyhalothrin**	00154801 **Acceptable/Guideline**	**Maternal NOAEL**: 10 mg/kg/day **Maternal LOAEL**: 30mg/kg/day (reduced body weight gain and food consumption). **Developmental NOAEL**: 30 mg/kg/day **Developmental LOAEL**: Not established
Lambda cyhalothrin	None	None
Gamma cyhalothrin	None	None
3-Generation Reproduction - Rat **Cyhalothrin**	00154802 **Acceptable / Guideline**	**Parental NOAEL**: 1.5 mg/kg/day **Parental LOAEL**: 5.0 mg/kg/day (decreased parental body weight and body weight gain during premating and gestation periods). **Reproductive NOAEL**: 5.0 mg/kg/day Reproductive LOAEL: Not established. **Offspring NOAEL**: 1.5 mg/kg/day **Offspring LOAEL**: 1.5 mg/kg/day (reduced pup weight and weight gain during lactation).
Lambda cyhalothrin	None	None
Gamma cyhalothrin	None	None
DERMAL TOXICITY STUDIES		
21-Day Dermal Toxicity - Rabbit **Cyhalothrin**	00154869 **Acceptable/Guideline**	**NOAEL**: 100 mg/kg/day **LOAEL**: 1000 mg/kg/day (significant weight loss)
Lambda cyhalothrin	None	None
Gamma cyhalothrin	None	None
21-Day Dermal Toxicity - Rat **Lambda** cyhalothrin	44333802 **Acceptable/Guideline**	**NOAEL**: 10 mg/kg/day **LOAEL**: 50 mg/kg/day (clinical signs of toxicity, decreased body weight and body weight gain)
Cyhalothrin	None	None
Gamma cyhalothrin	None	None
INHALATION TOXICITY STUDY		

Table 2: Comparative Toxicity Studies with Cyhalothrin, Lambda cyhalothrin and Gamma cyhalothrin

STUDY TYPE - ISOMER	MRID NO. CLASSIFICATION	RESULTS
21-Day Inhalation Toxicity - Rat **Lambda** cyhalothrin	41387702 **Acceptable/Guideline**	**NOAEL**: 0.08 mg/kg/day **LOAEL**: 0.90 mg/kg/day (clinical signs of neurotoxicity, decreased body weight gains, increased incidence of punctate foci in cornea, slight reductions in cholesterol in females, slight changes in selected urinalysis parameters).
Cyhalothrin	None	None
Gamma cyhalothrin	None	None
METABOLISM STUDIES		
Metabolism and Pharmacokinetics **Lambda cyhalothrin** **Cyhalothrin**	00151116, 00150852, 00150852, 00150852, 00153036, 00153037 **Acceptable/Guideline**	In the rat, approximately 55% of the oral dose is absorbed. It is extensively metabolized when absorbed. After subcutaneous administration, the urinary/fecal excretion ratio is 2.5:1.0. Over 50% of the dose remained in the carcass 7 days after a subcutaneous dose. Metabolism includes cleavage of the ester to cyclopropylcarboxylic acid and a phenoxybenzyl derivative. The distribution patterns and excretion rates in the multiple oral dose studies are similar to the single oral dose studies. There is accumulation of unchanged compound in the fat upon chronic administration. Otherwise, cyhalothrin is rapidly metabolized and excreted. Cyclopropyl carboxylic acid, 3-phenoxybenzoic acid, glucuronide conjugated 3-4'-hydroxyphenoxy benzoic acid and a sulfate conjugate were identified in the urine. Cyhalothrin is taken up slowly by the fat and released slowly. It is rapidly released by blood, kidneys, liver.

Table 3: Comparative Results of Mutagenicity Studies

	Test Compound/Dose levels			
Assay	**Gamma cyhalothrin**	**Lambda cyhalothrin**	**Cyhalothrin**	**Results**
S. typhimurium, 5 strains, ± S9	10-5000 µg/plate	1.6-5000 µg/plate	Up to 2500 µg/plate	All were Negative
In vitro chromosome aberration, ± S9	Rat lymphocytes, 45-1000 µg/ml	Human lymphocytes, 100-1000 µg/ml	N.D.	All were Negative
Mouse lymphoma L5178Y, ± S9	15.6-4000 µg/ml	125-4000 µg/ml	N.D.	All were Negative
In vivo bone marrow	Mouse, 0, 1, 2 or 4 mg/kg bw, oral	Mouse, 22 or 35 mg/kg bw, i.p.	Rat, 1.5, 7.5 or 15 mg/kg bw oral	All were Negative

B

CYHALOTHRIN MICRONUCLEUS [84-2]

EPA Reviewer: Irving Mauer, Ph.D. Date: 09/29/03
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 10/08/03
Toxicology Branch, HED (7509C)

TXR No.: 0051566

DATA EVALUATION RECORD

STUDY TYPE: *In vivo* mammalian cytogenetics - micronucleus assay in mice OPPTS 870.5395 [84-2], OECD 475.

DP BARCODE: D288067 SUBMISSION CODE: S28592

P.C. CODE: 128807 TOX. CHEM. NO.: None.

TEST MATERIAL (PURITY): G F-231 (a formulation containing 14.7% technical XR-225 a.i.).

SYNONYMS: None.

CITATION: Spencer, P.J., Hammond, T.A. and Beuthin, D.J. (2001). Evaluation of GF-231 in the Mouse Bone Marrow Micronucleus Test, performed at Toxicology and Environmental Research and Consulting, Dow Chemical Company, Midland (MI). Laboratory Project Study ID: 001190, dated 14 February 2001. MRID 45447401. Unpublished.

SPONSOR: Dow AgroSciences LLC, Indianapolis (IN).

EXECUTIVE SUMMARY:

In a cytogenetics (micronucleus) assay (MRID 45447401), groups of male mice (6/dose/group) were administered GF-231 (14.7% a.i., dissolved in distilled water, DW) by oral gavage on two consecutive days at doses of 50, 100 and 200 mg/kg/day, and sacrificed after the second dose. An additional group of six males was administered the highest dose, HDT (200 mg/kg/day), to replace animals that may die at this dose. Additional groups of six males were given consecutive doses of DW, or a single dose of the mutagen, cyclophosphamide (CP, 120 mg/kg), and sacrificed 24 hours after their last dose, to serve as vehicle (negative) and positive controls, respectively. At sacrifice, femoral bone marrow was examined for the presence of micronucleated polychromatic erythrocytes (MNPCEs); the frequencies of MNPCEs from treated animals were compared to vehicle controls.

In a preliminary range-finding test with both sexes, treatment-related toxicity (including death, incoordiantion, increased reactivity, convulsions and perioral soiling) was observed in test article-treated animals at the HDT (200 mg/kg). Evidence of comparable animal toxicity (but no mortality) was also found in the main mutagenicity assay in animals at the HDT of GF-231, but **no significant differences from vehicle controls in erythropoieses (% PCE) or MNPCE frequencies.** In contrast, the positive control group (CP) a manifested a marked induced increase in MNPCEs accompanied by a significant decrease in % PCE.

This study is classified as **acceptable/guideline**, and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vivo* cytogenetic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Control and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test Material:** GF-231 (an end-use formulation)

Description: Liquid.
Lot/Batch No.: N/A.
Purity: 14.7% a.i. (XR-225, cyhalothrin, as determined by HPLC).
Stability of compound: Not provided.
Solvent used: Distilled water (DW).
Other comments: None.

2. **Control Materials:**

Negative/Route of Administration: None.
Vehicle/Final Volume/route of Administration: DW/10 mL/kg/oral gavage.
Positive/Final dose(s)/Route of Administration: Cyclophosphamide/12 0 mg/kg/oral gavage.

3. **Test Compound Administration:**

Volume: 10 mL/kg
Route of Administration: Oral gavage/10 mL/kg.

Dose Levels:

a. Preliminary Dose Range-Finding Test (4 animals/sex/dose):
0, 50, 100, 200 mg/kg/day, for two days (24 hours apart).

b. Main Mutagenicity Assay (6 males/dose):
0, 50, 100, 200 mg/kg/day, for two days (24 hours apart).

4. **Test Animals:**

a. Species Mouse Strain CD-1(ICR)BR Age: 8 weeks

b. Number of male animals used per dose in the main assay: 6 males.
(No females.)

c. Properly maintained? Yes.

B. TEST PERFORMANCE:

1. **Treatment and Sampling Times:**

a. Test compound and vehicle (DW) control:

Dosing: ____once ___x___twice (24 hours apart)
____other (describe): None.

Sampling (after last dose): _____6 hr _____ 12 hr ___x___ 24 hr _____48 hr _____ 72 hr (mark all that are appropriate).

b. Positive control (CP):

Dosing: ___x___once ____twice (24 hr apart) _____other (describe): None.

Sampling (after last dose): ____6 hr ____12 hr ___x___24 hr _____48 hr _____72 hr

(Mark all that are appropriate.)

2. **Tissues and Cells Examined:**

___x___bone marrow _____other (list): None.

Number of polychromatic erythrocytes (PCE) examined per animal: _2000__.

Number of PCEs to normochromatic erythrocytes (NCE, *i.e.*, ratio of PCE/NCE, given as "% PCE") examined: 200 erythrocytes per animal .

3. **Details of Slide Preparation:** Conventional cytological procedure (same as MRID 45447326).

4. **Statistical Methods:**

Counts were first transformed by adding "one" to each individual MNPCE count, then taking the natural log. Transformed MNPCE and %

PCE data were analyzed by one-way ANOVA; pairwise comparisons by Dunnett's t-test. Alpha level was 0.05 for all methods.

5. **Evaluation Criteria**:

(Not provided).

6. **Body Temperature Data Collection**:

Animal temperature was monitored during the range-finding tests[1], using a programmable transponder (from BioMedic Data Systems, Seaford, DE), immediately prior to each dosing, about two to five hours after dosing, and prior to sacrifice. Body temperature changes have previously been reported to produce an increase in micronuclei (Asanami and Shimono, 1997[2]; Asanami and Shimono, 1998[3] as given in "REFERENCES", but printed in text, MRID 45447401, p. 14, as "Asanami *et al* ").

II. REPORTED RESULTS:

A. ANALYSES:

Analytically determined concentration of the test material in the dosing solutions for the main mutagenicity assay ranged from 85% to 102% of the targeted values (MRID 45447401, p. 34 - ATTACHMENT Table 13).

B. PRELIMINARY RANGE-FINDING TESTS (both sexes):

There were no significant changes in body weights or body temperatures at any treatment dose of GF-231 (MRID 45447401: pp. 21 to 24 - ATTACHMENT Tables 2 to 5; pp. 30 to 33 - ATTACHMENT Tables 9 to 12). Evidence of

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[1]Temperatures were not monitored in the main assay since, according to the investigators, no "significant" changes (greater than +1° or -3° C) were observed in the preliminary range-finder test.

[2]Asanami, S. and Shimono, K.: High temperature induces micronuclei in mouse bone marrow. Mutation Res., 390; 19-83 (1997).

[3]Asanami, S. and Shimono, K.: Transient hypothermia induces micronuclei in mice. Mutation Res., 413, 7-14 (1998).

pyrethroid toxicity (increased "reactivity," convulsions, perioral soiling) was observed at the HDT (200 mg/kg/dayJ), including one death, deemed by the investigators to be treatment-related (MRID 45447401, pp. 25 to 29 - ATTACHMENT Tables 6 to 8).

C. MUTAGENICITY ASSAY (only males evaluated[4]).

Slight indications of clinical toxicity were observed in two high dose males (perioral soiling in one, increased "reactivity" in another), but no effects on body weights (MRID 45447401, pp. 35 to 37 - ATTACHMENT Tables 14 to 16), ratio of bone marrow PCEs to NCEs (pp. 42, 43 - ATTACHMENT Tables 18, 19), frequencies of MNPCEs in GF-231 - treated animals compared to concurrent vehicle controls (pp. 42, 43 - ATTACHMENT Tables 18, 19), or to recent laboratory historical controls (p. 45 - ATTACHMENT Table 20). In contrast, the positive control (CP) group manifested a marked increase ($p \leq 0.05$) in MNPCEs, and a significant ($p \leq 0.05$) decrease in % PCE, indicative of bone marrow toxicity.

Therefore, the investigators concluded that GF-231 was considered negative in the mouse bone marrow micronucleus test.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. Although only minor adverse effects were found in the main micronucleus assay, evidence of moderate to severe clinical toxicity, including stated "treatment-related mortality", was reported in the preliminary range-finding test to be assured that a sufficiently high dose level of the test article was administered. Therefore, the EPA reviewers agree with the investigators' conclusions that GF-231 (a formulation containing 14.7% a.i.of technical XR-225) did not increase the frequency of micronucleated erythrocytes when administered by oral gavage up to a toxic level, and thus was cytogenetically negative in this mouse bone marrow micronucleus assay.

B. DEFICIENCIES:

None.

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[4]Since, as noted by the investigators, no differences in animal toxicity were found between GF-231-treated males and females in the range-finder test.

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CYHALOTHRIN BACTERIA/MAMMALIAN ACTIVATION; GENE MUTATION (84-2)

EPA Reviewer: Irving Mauer, Ph.D. Date: 09/29/03
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 10/08/03
Toxicology Branch, HED (7509C)

TXR No.: 0051566

DATA EVALUATION RECORD

STUDY TYPE: Bacterial system, *e.g.*, *Salmonella typhimurium-Escherichia coli*/mammalian activation gene mutation assay; OPPTS 870.5100 [84-2] OECD 471, 472.

DP BARCODE: D288062 SUBMISSION CODE: S628578

P.C. CODE: 128807 TOX. CHEM. NO.: None.

TEST MATERIAL (PURITY): GF-317 (a formulation containing 6% of XR-225 a.i.).

SYNONYMS: TSN 102331

CITATION: Mecchi, M.S. (2001). *Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay Preincubation Method with a Confirmatory Assay with GF-317, performed by Covance Laboratories (Covance), Vienna (VA). Covance Study No. 21846-0-422 OECD (Dow Study ID: 001220), dated March 29, 2001. MRID 45447402. Unpublished.

SPONSOR: Dow Chemical Company, Midland (MI), for Dow AgroSciences, Indianapolis (IN).

EXECUTIVE SUMMARY:

In independent repeat (initial and confirmatory) preincubation assays in bacteria (MRID 45447402), cultures of four histidine-deficient (*his*$^-$) strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and the tryptophan-deficient (*try*$^-$) strain of *Escherichia coli* WP2 *uvr*A were exposed to GF-317 (a formulation containing 6 % XR-225 a.i., dissolved in water, H_2O) at six concentrations ranging from 33.3 to 5000 µg/plate, in the presence and absence of exogenous metabolic activation (± S9) provided by hepatic microsomes from Aroclor 1254-treated rats. Additional cultures were exposed to water alone, or to strain-specific mutagens, to serve as vehicle and positive controls, respectively. Following plate incubation at 37 ± 2° C for 52 ± 4 hours, the numbers of revertant colonies (*his*$^+$, *try*$^+$) in test cultures were compared to those in vehicle controls.

No evidence of cytotoxicity (thinninng or absence of the background bacterial lawn and/or reduction in revertants) was observed at any dose up to the the limit, 5000 μg/plate, in any tested strain, either in the presence or absence of activation. Further, **the test compound did not induce any significant increase in revertants,** compared to marked increases induced by mutagen-treatment.

Therefore, GF-317 is considered non-mutagenic in the standard battery of bacterial strains.

This study is classified as **acceptable/guideline** and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test Material:** GF-317
Description: Opaque, white to off-white, viscous liquid.
Lot/Batch No.: NA
Purity: 6% a.i. XR-225 as the a.i.
Stability of compound: N/A.
CAS No.: N/A
Solvent used: Water (H_2O).
Other comments: Store at ambient temperatures.

2. **Control Materials:**

Negative: None.
Solvent/final concentration: H_2O/50 μL
Positive: **Non-activation:**

Sodium azide	2.0	µg/plate for TA100, TA1535
2-Nitrofluorene	1.0	µg/plate for TA98
ICR-191[1]	2.0	µg/plate for TA1537
Other (list): 4-NQO[2]	0.4	µg/plate for WP2 *uvr*A

Activation:

2-Aminoanthracene (2-anthramine)	2.5 2.5	µg/plate for µg/plate for	TA100, TA1535, TA1537 WP2 *uvr*A
Other (list):			
Benzo[a]pyrene	2.5	µg/plate for	TA98

[1]ICR-191 is: acridine, 6-chloro-9(3((2-chlorethyl)amino)propyl)amino-2-methoxy-dihydrochloride.

[2]4-NQO is: 4-nitroquinoline-N-oxide.

3. **Metabolic Activation**: S9, purchased from Molecular Toxicology, Inc., was derived from male Sprague-Dawley rats.

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobarbital		non-induced		mouse		lung
	none				hamster		other
	other						other

Describe S9-mix composition (prepared in-house):

Component	**Amount** (mL)
H_2O	0.70
IM NaH_2PO_4/Na_2HPO_4, pH 7.4	0.10
0.25 M Glucose-6-phosphate	0.02
0.10 M NADP	0.04
0.825 M KCl/0.2 M $MgCl_2$	0.04
S9 Homogenate	0.10
TOTAL	1.00

4. **Test Organisms**: *S. typhimurium* strains (from Dr. B Ames, UCal)

	TA97	x	TA98	x	TA100		TA102
	TA104	x	TA1535	x	TA1537		TA1538

List any others: *E. coli*, WP2 *uvr*A (from the National Collection of Industrial Bacteria, Torrey Research Station, Scotland, UK).

Properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. **Test Compound Concentrations Used:**

Cytotoxicity (Range-Finding) Tests (with TA100, WP2 *uvr*A ± S9; 1 plate per dose): 6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1000, 3330, 5000 µg/plate.

Main Assays:

Non-activated and activated conditions (all strains; 3 plates per dose per strain): 33.3, 100, 333, 1000, 3330, 5000 µg/plate.

B. TEST PERFORMANCE:

The assays employed the preincubation variation of the standard Ames Test,[3,4] as follows:

1. Test article, tester strain and the S9-mix (for activation), or 0.1 M phosphate buffer (for non-activation) were first incubated for 20 ± 2 minutes at 37 ± 2° C in a tube; then
2. molten selective overlay agar was added; and
3. agar and reaction components were mixed; after which
4. the tube mixture was overlayed onto a minimal agar plate (15 x 100 mm Petri dish).
5. After agar solidified, plates were inverted and further incubated at 37 ± 2°C for 52 ± 4 hours, at the end of which
6. revertant colonies were counted

All concentrations of the test article, vehicle and positive controls were plated in triplicate.

The condition of the background bacterial "lawn" was assessed both macro- and microscopically for evidence of cytotoxicity and test article precipitation. The number of revertant colonies per test plate for vehicle controls and test article-treated plates were counted manually. Revertant colonies per plate for positive

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[3]Yahagi, T. Degawa, M., Seino, Y., Matsunima, T., *et al.* "Mutagenicity of carcinogenic azo dyes and their derivatives." Cancer Letters 1: 91-96 (1975).

[4]Maron, D.M. and Ames, B. "Revised methods for the *Salmonella* mutagenicity test." Mutation Research 113: 173-215 (1983).

controls were counted by an automated colony counter (manufacturer and model not stated).

Criteria: Criteria for assay acceptance and response were both presented.

II. REPORTED RESULTS:

A. ANALYSES:

Results of three analytical procedures indicated percent of target concentration in the vehicle (H_2O) were ≥ 100% for cumulative doses ranging from 666 to 100,000 µg/mL for the first mutation assay and 116 to 72% (lowest dose) for 666 to 100,000 µg/mL for the confirmatory mutation assay.

B. PRELIMINARY CYTOTOXICITY (RANGE-FINDING) ASSAYS:

A slight reduction in background lawn was found in non-activated (-S9) cultures of TA100 at the two highest doses (3330 and 5000 µg/plate) in the first of two range-finding tests; however, this was not considered evidence of cytotoxicity, since it was not accompanied by a decrease in revertants, nor did it occur in activated test cultures of TA100, nor was observed in WP2 *uvr*A ± S9 (MRID 45447402, pp. 22 and 23 - ATTACHMENT Tables 1 and 2). Further, no precipitation of test article was observed at any dose.

C. MUTAGENICITY ASSAYS:

No cytotoxicity was found in either of two mutagenicity assays at any dose (up to the limit, 5000 µg/plate) in any test article-treated culture, in the presence or absence of S9-mix, and no precipitation of test article. Contamination in the second mutagenicity assay necessitated a repeat in a third assay with all strains.

There were no increases over concurrent water controls in revertant colonies in any valid assay, in contrast to marked increases induced in all positive control cultures (MRID 45447402, pp. 24 to 28 - ATTACHMENT Tables 3 to 6).

Therefore, the investigator concluded that GF-317 did not produce significant increases in revertant colonies either in a battery of *S. typhimurium* strains or in *E. coli* WP2 *uvr*A.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The EPA reviewers agree with the investigator that GF-317 (a formulation containing 6% of technical XR-225 as a.i.) was non-mutagenic in the conventional battery of bacterial strains treated up to limit dosing (5000 µg/mL).

B. STUDY DEFICIENCIES:

None.

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D

CYHALOTHRIN MICRONUCLEUS [84-2[

EPA Reviewer: Irving Mauer, Ph.D. Date: 09/29/03
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 10/08/03
Toxicology Branch, HED (7509C)

TXR No.: 0051566

DATA EVALUATION RECORD

STUDY TYPE: *In vivo* mammalian cytogenetics - micronucleus assay in mice; OPPTS 870.5395 [84-2]; OECD 475.

DP BARCODE: D288062 SUBMISSION CODE: S628578

P. C. CODE: 128807 TOX. CHEM. NO.: None.

TEST MATERIAL (PURITY): GF-317 (Lot No. EO757-41, an end-use formulation containing 5.9% XR-225 a.i.).

SYNONYMS: None.

CITATION: Spencer, P.J., Hammond, T.A. and Beuthin, D.J. (2001): Evaluation of GF-317 in the Mouse Bone Marrow Micronucleus Test, performed at Toxicology and Environmental Research and Consulting, Dow Chemical Company, Midland, MI. Laboratory Project Study ID: 001237, dated 26 March 2001. MRID 45447403. Unpublished.

SPONSOR: Dow AgroSciences LLC, Indianapolis, IN.

EXECUTIVE SUMMARY:

In a cytogenetics assay (MRID 45447403), groups of male mice (6/dose) were administered GF-317 (a formulation containing 5.9% technical XR-225 a.i., Lot No. EO751-41, dissolved in distilled water, DW) by oral gavage at two consecutive daily doses of 300, 600 and 1000 mg/kg/day, and sacrificed 24 hours after the second treatment. Additional groups of six males were given two consecutive doses of the vehicle (DW) alone, or a single dose of the mutagen, cyclophosphamide monohydrate (CP, 120 mg/kg), and sacrificed 24 hours after the last dose, to serve as negative and positive controls, respectively. At sacrifice, femoral bone marrow from each animal was processed for the evaluation of micronucleated polychromatic erythrocytes (MNPCEs). The frequencies of MNPCEs in bone marrow from treated animals were compared to DW controls.

In preliminary range-finding tests, most mice (both sexes treated) administered consecutive oral doses of GF-317 ranging from 100 to 1000 mg/kg/day showed major signs of moderate to severe clinical toxicity at ≥ 600 mg/kg/day, including one death (female) given 1000 mg/kg/day. There was, however, no evidence of an effect on body weight or body temperature.

In the main micronucleus assay (using males only[1]), comparable degrees of clinical toxicity were observed at ≥ 600 mg/kg/day, including the death of all six 600 mg/kg/day animals, as well as six males given 1000 mg/kg/day; all mortalities were deemed by the investigators to be treatment-related. Signs of pyrethroid toxicity was seen at 600 and 1000 mg/kg/day. No effects on body weights of test animals were found, and no significant differences in frequencies of MNPCEs between GF-317-treated groups and DW controls. By contrast, positive (CP) controls induced a marked increase in MNPCEs, and a significant decrease in % PCE.

Therefore, GF-317 is considered negative for cytotoxic activity (structural chromosome aberrations).

This study is classified as **acceptable/guideline** and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vivo* cytogenetic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

[1]Since, according to the investigators, there were no differences between sexes in clinical toxicology.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test Material**: GF-317
Description: Liquid
Lot/Batch No.: EO751-41
Purity: 5.9% XR-225 a.i.
Stability of compound: Stable, according to three analytical determinations using HPLC (MRID 45447403, pp. 22, 36 and 52 - Tables 1, 13 and 25).

CAS No.: (N/A)
Solvent used: Distilled water (DW).
Other comments: None.

2. **Control Materials**:

Negative/Route of Administration: None.
Vehicle/Final Volume/Route of Administration: DW/10 mL/kg/by oral gavage.
Positive/Final Dose(s)/Route of Administration: Cyclophosphamide monohydrate (CP)/120 mg/kg/oral gavage (dissolved in DW).

3. **Test Compound Administration**:

Volume: 10 mL/kg.

Route of Administration: Oral gavage.

Dose Levels:

a. Range-Finding Tests (4/sex/dose):

Test No. 1: 100, 200, 400 mg/kg/day – males and females.
Test No. 2: 600, 800, 1000 mg/kg/day – males and females.

b. Main Mutagenicity Assay (6 M/dose): 300, 600, 1000 mg/kg/day – males only.

4. **Test Animals** (males only in main assay)

a. Species mice Strain CD-1(ICR)BR Age 7 weeks
Weight Male ~ 29 g Female None - Micronucleus Assay.
Source: Charles River Laboratories, Portage (MI).

b. Number of animals used per dose: 6 Males No females.

c. Properly maintained? Yes.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

a. Test Compound and DW Control.

Dosing: _____once __x__twice (24 hours apart) other (describe):
None.

Sampling (after last dose): ____6 hr ____12 hr __x__24 hr

_____48 hr ____72 hr other (describe):

(Mark all that are appropriate.)

b. Positive Control:

Dosing: __x__once _____twice (24 hr apart) other (describe):

None.

Sampling (after last dose): _____6 hr _____12 hr __x__24 hr

_____48 hr _____72 hr Other (describe):

(Mark all that are appropriate.)

2. Tissues and Cells Examined:

__x__bone marrow _____other (list):

Number of polychromatic erythrocytes (PCE) examined per animal: 2000.

Ratio of PCE to normochromatic erythrocytes (NCE) examined in 200 erythrocytes, by:

$$\frac{\text{PCE}}{\text{PCE + NCE}} 100 \quad (= \% \text{ PCE})$$

Other (if other cell types examined, describe): None.

3. Details of Slide Preparation:

Bone marrow was obtained from both femurs; cells were suspended in serum and centrifuged. Cell pellets were prepared by conventional cytological procedure on microscope slides, and stained with Wright-Giemsa solution. Slides were coded prior to scoring.

4. Statistical Methods:

Raw counts of MNPCEs were first transformed (by adding "one" to each count), then obtaining the natural log of the adjusted numbers. Transformed MNPCE and percent PCE were analyzed separately by one-way ANOVA. Pair-wise comparisons of treated *vs.* control groups employed Dunnett's t-test, one-sided (upper) for MNPCE and two-sided for % PCE. Linear dose-related trends were performed if pair-wise comparisons were significant. Alpha level for all tests was 0.05.

5. Body Temperature:

The relative body temperatures of treated animals were monitored during the range-finding tests, using programmable transponders (BioMedic Data Systems, Seaford, DE), immediately prior to dosing, two to five hours after each dose, and prior to sacrifice. (Temperatures were not collected during the main study because no remarkable changes were observed

during the range-finders.) The investigators referred to studies reporting that "significant" decreases or increases (-3° / + 1° C) produce increased micronuclei in mice[2,3]

5. Evaluation Criteria:

Criteria for assay acceptance and response were both presented.

II. REPORTED RESULTS:

A. ANALYSES:

Concentrations of GF-317 in the vehicle (DW) in three separate analytical determinations ranged from 96% to 106% of target.

B. PRELIMINARY DOSE RANGE-FINDING TESTS (with both sexes):

In two tests employing consecutive daily doses ranging from 100 to 1000 mg/kg/day GF-317, no changes in body temperature or appreciable changes in body weight of males or females were observed (MRID 45447403, pp 23, 24, 32, 33, 37, 38, 48, 49 - ATTACHMENT Tables 2, 3, 9, 10, 14, 15, 21, 22). Most test article-treated mice (equal number of males and females) manifested clinical toxicity (incoordination, "reactivity", convulsions, and perioral soiling) at ≥ 600 mg/kg/day, including one treatment related-death (a female) given 1000 mg/kg/day. Since there was no "remarkable" change in either body temperature or body weight, only male mice were treated in the micronucleus assay.

C. MICRONUCLEUS ASSAY (only males evaluated):

Body weights in treated animals were unaffected (MRID 45447403, p. 53 - Table 26), but death occurred on Day 2 in all six 600 mg/kg/day animals and two given 1000 mg/kg/day. Both mid- and high-dose males showed clinical signs of pyrethroid toxicity as described in the range-finding tests.

However, at no dose were significant differences found in MNPCE frequencies or

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[2]Asanami, S. and Shimoko, K. (1997). High Body Temperature Induces Micronuclei in Mouse Bone Marrow. Mutation Res., 390, 79-83.

[3]Asanami, S. and Shimoko, K. (1998). Transient Hypothermia Induces Micronuclei in Mice. Mutation Res., 413, 7-14.

% PCE between GF-317-treated animals and concurrent DW controls (MRID 45447403, p. 65 - ATTACHMENT Table 30) or the laboratory historical control values (p. 67 - ATTACHMENT Table 32). In contrast, the positive control (CP) showed a significant ($p < 0.05$) increase in MNPCE, and a decrease ($p < 0.05$) in % PCE.

Therefore, the investigators concluded that GF-317 was inactive in increasing the frequencies of MNPCEs over vehicle controls up to the maximum tolerated dose.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The EPA reviewers agree with the investigators' conclusions that GF-317 (an end-use formulation containing 5.9% of technical XR-225, cyhalothrin) was cytogenetically inactive when assayed in the mouse bone marrow micronucleus test up to a maximum tolerated clinical dose.

B. DEFICIENCIES: None.

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E

CYHALOTHRIN MAMMALIAN CELLS IN CULTURE; GENE MUTATION [84-2]

EPA Reviewer: Irving Mauer, Ph.D. Date: 11/07/03
Registration Action, Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 10/28/03
Toxicology Branch, HED (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Mammalian cells in culture gene mutation assay in mouse lymphoma (L5178Y) cells; OPPT 870.5300 [84-2]; OECD 476.

DP BAR CODE: D288053 SUBMISSION CODE: S625041

P. C. CODE: 128807 TOX. CHEM. NO.: None.

TEST MATERIAL (PURITY): XR-225 (Lot No. CH519JMi87ABISE, TSN101800, 100% a.i.).

SYNONYMS: X 670225. Chemically: Cyclopropanecarboxylic acid, 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl-, cyano(3-phenoxyphenyl)methyl ester, [IR-[1. alpha. (S*),3.alpha.(Z)]] - ; cyhalothrin.

CITATION: Linscombe, V.A., Schisler, M.R. and Beuthin, D.J. (2000). Evaluation of XR-225 in the Mouse Lymphoma (L5178Y $TK^{+/-}$) Forward Mutation Assay, performed at Toxicology and Environmental Research and Consulting, Dow Chemical Company, Midland, MI. Laboratory Project Study ID: 991071, dated 27 September 2000. MRID 45447404. Unpublished.

SPONSOR: Dow AgroSciences (DAS) LLC, Indianapolis (IN).

EXECUTIVE SUMMARY:

In independent repeat mammalian cell forward gene mutation assays (MRID 45447404), cultures of mouse lymphoma L5178Y cells, heterozygous at the thymidine kinase locus ($TK^{+/-}$), were exposed for four hours to XR-225 (Lot No. CH519JMi87ABISE, TSN 101800, 100% a.i., dissolved in dimethyl sulfoxide, DMSO), in the presence and absence of exogenous metabolic activation provided by hepatic microsomes from Aroclor 1254-treated rats (± S9), at concentrations ranging from 3.9 to 1000 µg/mL +S9, and from 156 to 4500 µg/mL -S9.

In addition to cultures exposed to the solvent (DMSO), other cultures were treated with the mutagens, methyl methanesulfonate (MMS) and 20-methylcholanthrene, the latter chemicals to serve as positive controls for the non-activated and activated test series. At harvest, forward mutation ($TK^{-/-}$) was

assessed by counting the numbers of colonies resistant to the analog of the nucleic acid base, pyrimidine, 6-trifluoropyrimidine (6-TFT), which permits only mutants to survive.

In preliminary cytotoxicity tests, moderate to severe decreases in relative suspension growth (RSG) were evident in non-activation cultures treated at ≥ 70.3 µg/mL ± S9, but beginning at lower concentrations, ≥ 12.5 µg/mL, in activated cultures. Addition of test material also resulted in cloudiness of the treatment medium at ≥ 70.3 µg/mL, with increasing precipitation at ≥ 1125 µg/mL. In the main mutagenicity assays, excessive toxicity (decreasing plating efficiencies) was evident in activated cultures exposed to ≥125-250 µg/mL, but only moderate toxicity was found in non-activated cultures exposed to higher doses (2000-4500 µg/mL). Precipitation was evident at ≥ 1000 µg/mL.

At no dose in any assay, ± S9, however, did XR-225-treated cultures manifest increased frequencies in mutant colonies over concurrent negative (solvent) controls, or background control data. In contrast, both positive controls displayed marked increases in mutant colonies.

Therefore, XR-225 is considered non-mutagenic in this mouse lymphoma forward mutation assay at the thymidine kinase locus.

This study is classified as **acceptable/guideline** and satisfies the FIFRA Test Guideline for *in vitro* mammalian gene mutation data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test Material**: XR-225.
Description: Off-white solid.
Lot/Batch No.: CH519JMi87ABISE, TSN101800.
Purity: 100% a.i.
Stability of compound: Stable (see Section II.A., below).
CAS No.: 76703-62-3.
Solvent used: Dimethyl sulfoxide (DMSO).
Other comments: None.

2. **Control Materials**:
Negative: None.
Solvent/final concentration: DMSO/1%.
Positive: Non-activation (concentrations, solvent): Methyl methanesufonate/10, 15 µg/mL/phosphate-buffered saline..

Activation (concentrations, solvent): 20-Methylcholanthrene/2.5, 5 and 7.5 µg/mL/DMSO.

3. **Metabolic Activation**:

S9, purchased from [an unnamed] "commercial" source, was prepared from hepatic homogenates (microsomes) derived from Aroclor 1254-treated male Sprague-Dawley rats.

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobarbital		non-induced		mouse		lung
	none				hamster		other
	other				other		

If other, describe below: None.

Describe S9-mix composition (prepared in-house):

Component	Amount
NADP	2.0 mg/mL
Sodium citrate	11.25 mg/mL
S9	5% (v/v)

Final concentration of S9-mix was 2%.

4. **Test Cells**: mammalian cells in culture.

__x__ Mouse lymphoma L5178Y cells ($TK^{+/-}$ -3.7.2 C clonal line).

_____ Chinese hamster ovary (CHO) cells.

_____ V79 cells (Chinese hamster lung fibroblasts).

_____ Other (list):

Source: Dr. Donald Clive, Burroughs Wellcome Laboratories, Research Triangle Park (NC).

Properly maintained? Yes.

Periodically checked for Mycoplasma contamination? Yes.

Periodically checked for karyotype stability? Not stated.

Periodically "cleansed" against high spontaneous background? Yes.

Media:

a. Maintenance: Fischer's (F_{10P}) containing 204 m/mL L-glutamine, 10% (v/v) heat-inactivated horse serum, 20 IU/mL penicillin, 20 µg/mL streptomycin sulfate, 0.22 mg/mL sodium pyruvate, and 10 mg/mL Plurionic F68

b. Treatment: F_{10P} without horse serum (in addition to cell-free "conditioned" medium).

c. Cloning: F_{10P} containing 0.28% granulated agar.

d. Selection: Cloning medium, supplemented with 1 µg/mL trifluorothymidine (TFT).

5. **Locus Examined:**

___x___thymidine kinase (TK).

Selection agent: _____bromodeoxyuridine (BrdU).

(give concentration) _____fluorodeoxyuridine (FdU).

___1 µg/mL___trifluorothymidine (TFT).

_____hypoxanthine-guanine-phosphoribosyl transferase (HPRT)

Selection agent: _____8-azaguanine (8-AG)

(give concentration) _____ 6-thioguanine (6-TG).

_____Na^+/K^+ ATPase

Selection agent: _____ Oubain

(give concentration)

_____Other (locus and/or selection agent; give details): None.

6. **Test Compound Concentrations Used**

a. Preliminary Cytotoxicity Tests (single doses).

Test No. 1: 17.6, 35.2, 70.3, 140.6, 281.25, 562.5, 1125, 2250, 4500 µg/mL ± S9..

Test No. 2: 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 100, 500 µg/mL +S9.

b. Main Gene Mutation Assays (duplicate doses):

Assay No. 1: 15.6, 31.5, 62.5, 125, 250, 500, 1000, 2000, 4500 µg/mL -S9

3.9, 7.8, 15.6, 31.25, 62.5, 125, 250, 500, 1000 µg/mL +S9

Assay No. 2, Trial I: 15.6, 31.25, 62.5, 125, 250, 500, 1000, 2000, 4000 µg/mL -S9

3.9, 7.8, 15.6, 31.25, 62.5, 125, 250, 500 µg/mL +S9

Assay No. 2 Trial II: 15.6, 31.25, 62.5, 125, 250, 500, 1000, 2000, 4000 µg/mL -S9

3.9, 7.8, 15.6, 31.25, 62.5, 125, 250, 500 µg/mL +S9

B. TEST PERFORMANCE:

1. PRELIMINARY CYTOTOXICITY TESTS/CELL COUNTS AND INDICES

". . . . . .After termination of treatment, the cells were rinsed to remove the test material and incubated in F_{10P}^-. At approximately 24 hours after treatment (Day 1), the test cultures were counted and diluted to a concentration of approximately 3×10^5 cells/ml with fresh F_{10P}^-. If the treated cells failed to multiply to a density of 4×10^5 on the first day following treatment, the culture was returned to the incubator without any dilution. On Day 2, cultures were again counted for cell density. From these cell counts, the following indices were calculated" [MRID 45447404, p. 15]:

$$\text{Day 1 SG = Suspension Growth over the first day} = \frac{\text{No. of cells per mL on Day 1}}{3 \times 10^5 \text{ cells/mL}}$$

$$\text{Day 1 RSG = Day 1 Relative Suspension Growth} = \frac{\text{Day 1 SG of treated}}{\text{Average Day 1 SG of negative control}}$$

Day 2 SG = Suspension Growth during the second day = $\frac{\text{No. of cells/mL on Day 2}}{3 \times 10^5 \text{ cells/mL (or the previous day cell no. if no adjustment was made on the previous day).}}$

CSG = Cumulative Suspension Growth during the first two days = Day 1 SG x Day 2 SG

Day 2 RSG (%) = Cumulative relative suspensions growth over the first two days =

$$\frac{\text{Cumulative suspension growth of treated culture during the first two days} \times 100}{\text{Average cumulative suspension growth of negative cultures during first 2 days}}$$

2. GENE MUTATION ASSAYS

a. Cell Treatment (in summary):

(1) Cells exposed to test compound, negative/solvent or positive controls for 4 hours (non-activated) 4 hours (activated).

(2) After washing, cells were cultured for 2 days (expression period) before cell selection.

(3) After expression, 3×10^6 cells/dish (3 dishes/group) were cultured for 12 days in selection medium to determine numbers of mutants; and 200 cells/dish (3 dishes/group) were cultured for 12 days without selective agent to determine cloning efficiency.

b. Cell Counts/Indices (in detail):

"Twenty-four hours following treatment (Day 1), test cultures were diluted to a concentration of 3×10^5 cells/mL with fresh F_{10P}. On Day 2 (48 hours following treatment), cultures with desired levels of survival were selected for cloning; cultures with < 15% Day 2 RSG were not cloned. A total sample size of 3×10^6 cells from each culture was suspended in cloning medium with trifluorothymidine (TFT), and plated into three Petri dishes (100 mm), allowed to gel for approximately 15 min. at 0-6° C, and returned to the incubator for approximately 12 days to allow for mutant colony formation. The cloning efficiency was determined by serially diluting the sample in cloning medium without TFT, and then plating the

cells into three Petri dishes (100 mm) at a concentration of approximately 200 cells per dish. The dishes were returned to the incubator for approximately 12 days before counting the number of colonies per dish.

"An image analyzer (LAI High-Resolution Colony Counting System, Loats Associates, Inc., Westminister, MD) was used to count and size colonies. Colonies that were ≥ 0.55 mm were classified as "large" colonies while those smaller than this were classified as "small" colonies.

"Relative total growth (RTG) was used to determine the cytotoxicity of various treatments. Calculations for RTG are described below." [MRID 45447404, pp. 15, 16].

$$\%RTG = \text{Day 2 RSG} \times \text{relative cloning efficiency (RCE)} \times 100$$

$$RCE = \frac{\text{Cloning efficiency of the treated at the time of mutant selection}}{\text{Average cloning efficiency of the negative control at the time of mutant selection}}$$

$$\text{Cloning Efficiency (CE)} = \frac{\text{Average no. of colonies in the TFT-free plates}}{\text{No. of cells seeded per plate}}$$

The mutant frequency (MF) per 10^6 clonable cells was calculated as follows:

$$\frac{\text{Average no.of mutants per TFT plate}}{\text{Cloning Efficiency}}$$

Mutant Index (MI) was calculated as follows:

$$\frac{\text{Mutant frequency of the treated culture}}{\text{Average mutant frequency of the negative control}}$$

c. Statistical Methods:

The frequency of total mutants per 10^6 clonable cells was statistically evaluated using a weighted analysis of variance; weights were derived from the inverse of the mutation frequency variance. The actual plate counts were assumed to follow

a Poisson distribution, therefore the mean plate count was used as an estimate of variance (Kirkland, 1989[1]).

A linear trend test and lack of fit test were employed (alpha ≤ 0.05) as omnibus tests to compare treated groups to the negative control. If there was a significant increasing trend or a significant lack of fit, a Dunnett's test was conducted, comparing each treated group and the positive control to the negative control (alpha ≤ 0.05, one-sided). The lack of fit test was merely an indicator that further analysis was required (*i..e.*, the Dunnett's test). An additional comparison of the positive control to the negative control (alpha ≤ 0.05) was conducted using a linear contrast statement.

d. Evaluation Criteria:

Criteria for assay acceptance and response were both presented.

For an assay to be acceptable, the mutation frequency in positive controls should be significantly higher than the negative controls. An additional criterion is that the mutation frequency in the negative controls should be within the limits of the laboratory historical control values and literature values. Assays with less than 50% average absolute cloning efficiency in the negative controls were only accepted after critical review.

Mutant frequencies were evaluated based upon statistical analysis as well as a number of other criteria (Clive *et al.*, 1995[2]). The test chemical is considered positive when the conditions listed below are met.

(1) There is a statistically significant, dose-related, reproducible increase in mutant frequency with a range of dose levels yielding > 20% relative total growth.

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[1]Kirkland, D. J. (editor) (1989). Statistical Evaluation of Mutagenicity Test Data, Cambride University Press, New York, N.Y., p. 78-87.

[2]Clive, D., Bolcsfoldi, G., Clements, J., Cole, J. *et al.* (1995). Consensus Agreement Regarding Protocol Issues Discussed During the Mouse Lymphoma Workshop: Portland, Oregon, May 7, 1994. Environmental and Molecular Mutagenesis 25: 165-168.

(2) The mutant frequency in at least one dose level of the treated cultures (resulting in > 20% relative total growth) is 100 x 10^{-6} above concurrent solvent controls (assuming these to be in the range of 20-80 x 10^{-6}).

The investigators also noted that the biological and scientific plausibility of the observed response is taken into consideration in the final interpretation of the data.

II. REPORTED RESULTS:

A. ANALYSES:

In repeat (three) analytical determinations using HPLC/UV, concentrations of XR-225 in stock solutions of DMSO ranged from 72% to 116% of target. There was no appreciable change in the pH and only a slight change in the osmolality of the culture medium following addition of the test material, as compared to culture medium with solvent alone.

B. PRELIMINARY CYTOTOXICITY ASSAYS:

In the first test at 9 concentrations ranging from 17.6 to 4500 µg/mL, moderate and alternating degrees (20% to 60%) of cytotoxicity (as measured by Day 2 RSG) were observed in non-activated (-S9) cultures, but cytotoxicity was more severe (≤ 10% RSG) in activated cultures (+S9) beginning at 35.2 µg/mL (MRID 45447404, pp. 23, 24 - ATTACHMENT Tables 1A, 1B). A second test under activation at a lower range of concentrations (0.78 to 500 µg/mL) revealed a more-or-less "straight-line" (dose-related) degree of cytotoxicity, from 71.5% Day 2 RSG at ≥ 12.5 µg/mL, to about 10% at the HDT (MRID 45447404, p. 25 - ATTACHMENT Table 1C). Increasing degrees of precipitation were also recorded in the initial test at dose levels ≥ 1125 µg/mL, but only "cloudiness" was observed at 500 µg/mL in the second test.

C. MAIN MUTAGENICITY ASSAYS:

Some of the main assays were considered by the investigators to be unacceptable for an appropriate assessment of mutagenicity, because of low absolute plating efficiencies (< 50%) of the solvent controls (MRID 45447404, pp. 30-33 - ATTACHMENT Tables 3A to 3D).

In the first series of assays considered acceptable, cytotoxicity (as determined by RTG, *i.e.*, % survival) ranged from 10% to 12% at the HDT (4000, 4500 µg/mL -S9; 1000 µg/mL +S9) (MRID 45447404, pp. 26, 28, 30, 32 - Tables 2A, 2C 3A, 3C). In

subsequent assays, cytotoxicity was moderately severe (26-28%) in non-activated cultures (-S9) up to the HDT (4000 µg/mL), but considered "excessive" in activated cultures at ≥ 125 µg/mL (MRID 45447404, pp. 34, 36, Tables 4A, 4C). The treatment medium became cloudy when ≥ 125 µg/mL test article was added, and increasing precipitation was evident at ≥ 1000 µg/mL ± S9.

However, at no dose was a significant increase in mutants (represented by TFT-resultant colonies) over concurrent solvent controls found either in the presence or absence of metabolic activation. All MFs in test article-treated cultures were within the laboratory's negative historical control values (MRID 45447404, p. 41. ATTACHMENT Table 6). In contrast, positive control cultures manifested marked increases in mutant colonies.

Therefore, XR-225 (cyhalothrin) was considered negative for gene mutation in this test up to cytotoxic precipitating dose levels..

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The EPA reviewers agree with the investigator's conclusion that cyhalothrin demonstrated no induced mutagenic activity up to cytotoxic/precipitating dose levels..

B. STUDY DEFICIENCIES:

None.

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F

CYHALOTHRIN *IN VITRO* CHROMOSOME ABERRATION OR SCE (84-2)

EPA Reviewer: Irving Mauer, Ph.D. Date: 11/07/03
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Nancy E. McCarroll Date: 10/23/03
Toxicology Branch, HED (7509C)

TXR No.: 0051566

DATA EVALUATION RECORD

STUDY TYPE: In Vitro chromosome aberration test; OPPTS 5375 [84-2]; OECD 473.

DP BAR CODE: D288053 SUBMISSION CODE: S625041

P. C. CODE: 128807 TOX. CHEM. NO.: None.

TEST MATERIAL (PURITY): XR-225 (Lot No. CH519JMi87ABISE; TSN 101800 - 100%)

SYNONYMS: X670225. IUPAC name: Cyclopropanecarboxylic acid, 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2.2-dimethyl-, cyano(3-phenoxyphenyl)methyl ester, [IR-[1.alpha .S*),3.alpha. (Z)]] -

CITATION: Linscombe, V. A., Jackson, K.M., and Engle, K. E. (2000). Evaluation of XR-225 in an *In Vitro* Chromosomal Aberration Assay Utilizing Rat Lymphocytes, performed at Toxicology and Environmental Research Consulting, The Dow Chemical Company, Midland, MI. Laboratory Project Study ID: 991072, dated 16 May 2000. MRID 45447405. Unpublished.

SPONSOR: Dow AgroSciences (DAS) LLC, Indianapolis, IN.

EXECUTIVE SUMMARY:

In independent, repeat (initial and confirmatory) assays (MRID 45447405), primary lymphocyte cultures from male rats were exposed for four hours to XR-225 (100% a.i., Lot No. CH519JMi87ABISE, TSN 101800, dissolved in dimethyl sulfoxide, DMSO), in the presence and absence of hepatic homogenates (± S9 microsomes) derived from Aroclor 1254-treated male rats, to seven concentrations ranging from 4.5 to 4500 µg/mL, and harvested 20 hours later (Initial Assay-1). Based upon mitotic indices (MI), cultures treated at 45, 150 and 450 µg/ml -S9 and 45, 450 and 1500 µg/mL +S9 were selected for the determination of structural (breaks and exchanges) and numerical (polyploid) chromosomal aberrations. In a confirmatory trial (Assay-2), non-activated (-S9) cultures were exposed continuously for 24 hours to nine concentrations of XR-225 ranging from 1.0 to 500 µg/mL, or at a slightly narrower range of 12 concentrations from 1.25 to 200 µg/mL in a repeat of Assay -2, whereas activated (+S9) cultures

were treated for 4 hours at six concentrations ranging from 15 to 3000 µg/mL; this series was also harvested at 24 hours. Based on reduced MIs and/or compound insolubility, non-activated cultures treated at 5, 20 and 40 µg/mL and activated cultures treated at 45, 150 and 1500 µg/mL were selected for determining chromosomal aberrations. In addition to cultures exposed to DMSO alone, others were treated with the mutagenic clastogens (chromosome-breakers) mitomycin C (MMC:0.05, 0.075, 0.5 µg/mL) and cyclophosphamide (CP:4 or 6 µg/mL), to serve as solvent (negative) controls (DMSO), and the latter as positive controls for the non-activated and activated test series, respectively.

Evidence of test substance bone marrow cytotoxicity (represented by decreases in MI) was observed in both non-activated and activated cultures, beginning at concentrations between 30 and 45 µg/mL, with dramatic decreases at ≤ 100 µg/mL, accompanied by increasing compound precipitation ≤ 450 µg/mL -S9 in Assay-1, and at 200-500 µg/mL ± S9 in the confirmatory assay. **At no concentration of X4-225 in either series of assays, however, were significant increases over concurrent solvent control or historical background values) in either structural or numerical aberrations found.** In contrast, marked and significant increases were induced in structural (but not numerical) aberrations for both positive controls.

Hence, XR-225 is considered negative for clastogenicity in this *in vitro* chromosomal aberration study in rat lymhocytes.

This study is classified as **acceptable/guideline** and satisfies the requirement for FIFRA Test Guideline (*in vitro* cytogenetics) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidential statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: XR-225
   Description: Off-white solid
   Lot/Batch No.: CH519JM:87ABISE, TSN 101800.
   Purity: 100% a.i.
   Stability of compound: Stable (see SECTION II.A., below).
   CAS No.: 76703-62-3
   Solvent used: Dimethyl sulfoxide (DMSO)
   Other comments: None.

2. Control Materials:
   Negative: None.
   Solvent/final concentration: DMSO/1%
   Positive: **Non-activation (concentrations, solvent):** Mitomycin C (MMC: 0.05, 0.075, 0.5 µg/mL; dissolved in culture medium).

   Activation (concentrations, solvent):
   Cyclophosphamide (CP: 4 and 6 µg/mL; dissolved in culture medium).

3. Activation: S9 (purchased from Molecular Toxicology, Inc., NC), was derived from Aroclor 1254-treated male Sprague-Dawley rats.

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobarbital		non-induced		mouse		lung
	none				hamster		other
	other						other

If other, describe below: None.

Describe S9-mix composition (prepared in-house):

Component	Amount (mM)
$MgCl_2$. 6 H_2O	10
Glucose-6-phosphate	5
NADP	4
$CaCl_2$	10
KCl	30
Sodium phosphate	50

Reconstituted S9-mix contained 2% (v/v) S9.

4. Test Compound Concentrations Used:

a. Preliminary Cytotoxicity Tests:

Initial (Assay-1) : 4.5, 15, 45, 150, 450, 1500, 4500 µg/mL ± S9

Confirmatory (Assay-2): 1.0, 3.3, 10, 33, 100, 200, 300, 400, 500 µg/mL -S9
15, 45, 150, 450, 1500, 3000 µg/mL +S9

Repeat (Assay-2): 1.25, 2.5, 5.0, 10, 20, 30, 40, 60, 80, 100, 150, 200 µg/mL - S9

Main Cytogenetic Assays:

Initial: 45, 150, 450 µg/mL ± S9
Confirmatory: 5, 20, 40 µg/mL -S9
45, 150, 1500 µg/mL +S9

5. Test Cells: Primary rat lymphocyte cultures were harvested from blood samples of male Sprague-Dawley rats. Lymphocytes were cultured in RPMI medium (with 25 mM HEPES supplemented with 10% heat-inactivated fetal bovine serum, antibiotics, antimycotics, and 2 mM L-glutamine, and 20 µg/mL phytohemagglutinin, PHA) for 48 hours at 37°C.

Properly maintained? Yes.

Cell line or strain periodically checked for Mycoplasma contamination? (Not needed.).

Cell line or strain periodically checked for karyotype stability? (Not needed.).

B. TEST PERFORMANCE:

1. Preliminary cytotoxicity Assay: [see Section I.A.4., above].

2. Cytogenetic Assay: [see Section 1.A.4., above.]

a. Cell Treatment:
(See Section 1.a.4, above.]

b. Treatment Medium: RPMI 1640, with HEPES and antibiotics, plus mitotic-stimulated phytohemaglutinin, PHA (but without serum).

c. pH/Osmolality: No changes in either pH or osmolality were found in treated cultures up to the limit concentration of XR-225 (= 10 mM) compared to cultures treated with DMSO alone.

d. Spindle Inhibition:

Inhibition used/concentration: Colcemid/0.2 µg/mL.

Administration time: 3 hours (before cell harvest)

e. Cell Harvest:

Treatment Time: Non-activated: 4 and 24 hours continuous.
S9-activated: 4 hours.

Cells exposed to test material, solvent or positive control were harvested : 20 [Assay-1]/ 0 [Assay-2] hours after termination of treatment [non-activated], 20 [Assay-1] 20 [Assay-2] hours after termination of treatment [activated],

f. Details of Slide Preparation:

Conventional cytological procedure (0.075 M KCl; methanol:acetic acid; Giemsa).

g. Metaphase Analysis:

No. of cells examined per dose: 200 for structural; 100 for numerical abnormalities: 1000 cells/replicates were examined to determine the mitotic index (MI)..

Scored for structural: Yes.

Scored for numerical: Yes. (Polyploidy)

Coded prior to analysis: Yes.

h. Evaluation criteria:

Criteria for both assay acceptance and response were both presented.

i. Data Compiled:

Cells with aberrations/cell =

$$\frac{\text{Total aberrations (excluding gaps, miscellany and severe damage)}}{\text{No. of cells scored}}$$

j. Statistical Analysis:

Statistical significance at $p \leq 0.05$ using overall Chi-Square and linear trend for structural aberrations, and Fisher's Exact Probability Test for polyploidy.

II. REPORTED RESULTS:

A. ANALYSIS:

Three analytical determinations using HPLC /UV found concentrations in DMSO to be 79% to 118% of target (MRID 45444705, pp. 21 to 23 - ATTACHMENT

Tables 1 to 3). It is of note that the lowest prepared concentrations for Assay 1 had the lowest achieved concentrations. All other levels contained ± 90% of the targeted concentrations.

B. PRELIMINARY CYTOTOXICITY TESTS:

Dose-related reductions in MIs (evidence of erythropoietic cytotoxicity) were found in both tests up to the limit dose, 4500 µg/mL (= 10 mM for this test substance), accompanied by increasing precipitation at ≥ 150 µg/mL, (MRID 45447405, pp. 24, 25, 29, 30, 31 - ATTACHMENT Tables 4A, 4B, 7A, 7B, 7C). Based on these findings, concentrations of 45, 150 or 450 µg/mL -S9; 45, 450 or 1500 µg/mL +S9 – Assay 1, or 5, 20 or 40 µg/mL -S9; 45, 150 or 1500 µg/mL +S9 – Assay 2 – were selected for evaluation of structural and numerical chromosome aberrations

C. MAIN CYTOGENETIC ASSAYS:

At no concentration in either series of trials of XR-225 (initial and/or confirmatory) were significant increases in cells with structural or numerical aberrations over solvent controls evident, either in the presence or absence of metabolic activation (MRID 45447405, pp. 26, 27, 28, 32, 33, 34 - ATTACHMENT Tables 5, 6A, 6B, 8, 9A, 9B); all test article-induced structural aberrations were within the laboratories' historical aberration frequencies (MRID 45447405, p. 35 - ATTACHMENT Table 10). In contrast, both positive controls induced marked increases in structural (but not numerical) aberrations.

Therefore, the investigators concluded that technical XR-225 was negative for either clastogenicity (structural aberrations) or polyploidy in this study.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The EPA reviewers agree with the investigators' conclusions that technical XR-225 (cyhalothrin) was negative for *in vitro* clastogenicity or polyploidy in repeat assays in rat lymphocytes up to cytotoxicity/precipitating concentrations.

B. DEFICIENCIES:

None.

454,476-05

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
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gamma-Cyhalothrin Tox review 0051566

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DATA EVALUATION RECORD

XR-225 (GAMMA CYHALOTHRIN)

Study Type: §82-1a, Subchronic Oral Toxicity Study in Rats

Work Assignment No. 01-01-03 B and A (Formerly 5-01-215 B and A)
(MRIDs 45447322 and 45447321)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by
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Signature: Steven Brecher
Date: 9/9/03

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Jess Rowland **Signature:** Jess Rowland
Science Information Management Branch, HED (7509C) **Date:** 3/15/04
Work Assignment Manager: PV Shah, Ph.D. **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509C) **Date:** 3/16/04

TXR#: 0051566

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity [feeding] - rat; OPPTS 870.3100a [§82-1a]; OECD 408.

PC CODE: 128807 **DP BARCODE:** D288053, D288062, and D288067
SUBMISSION NO.: S625041, S628578, and S628592

TEST MATERIAL (PURITY): XR-225 (Gamma cyhalothrin; 100% a.i.)

SYNONYMS: X670225, cyclopropanecarboxylic acid, 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl-cyano(3-phenoxyphenyl)methyl ester, [1R-[1.alpha.(S*),3.alpha.(Z)]]-

CITATION: Johnson, K.A., M.D. Dryzga (2000) XR-225: 13-week repeated dose dietary toxicity and 4-week recovery studies in Wistar Han rats. Toxicology & Environmental Research and Consulting, Midland, MI. Laboratory Project Study Id.: 991191, December 27, 2000. MRID 45447322. Unpublished.

Johnson, K.A. T.K. Jeffries, S.J. Day, *et al.* (1999) XR-225: 4-week repeated dose dietary toxicity study in Wistar Han rats. Toxicology & Environmental Research and Consulting, Midland, MI. Laboratory Project Study Id.: 991089, October 6, 1999. MRID 45447321. Unpublished.

SPONSOR: Dow AgroSciences (DAS) LLC, 9330 Zionsville Rd., Indianapolis, IN

EXECUTIVE SUMMARY - In a subchronic oral toxicity study (MRID No. 45447322), XR-225 (gamma cyhalothrin; 100% a.i.; Lot/Reference # CH519JMi87ABISE, TSN101800) was administered to 10 Wistar Han rats/sex/group in the diet at dose levels of 0, 2.5, 10, 50, or 100 ppm (equivalent to 0/0, 0.2/0.2, 0.7/0.8, 3.4/4.2, and 6.6/8.0 mg/kg/day in males/females) for 13 weeks. Additionally, 10 Wistar rats/sex/group were similarly treated at 0 and 100 ppm for 13 weeks and then observed during a recovery period of 4 weeks.

No treatment-related adverse effects were observed for body weight, body weight gain, food consumption, ophthalmology, hematology, clinical chemistry, mixed function oxidase activity, or urinalysis at dose levels of 2.5, 10, or 50 ppm. Minor decreases in body weight/body weight gain were observed at 100 ppm, but were not considered to be treatment-related.

At 100 mg/kg/day, the target organs appear to be the skin and neuromuscular system. Mortality in high dose males (3/20) was considered to be treatment-related. At 100 ppm, animals showed clinical signs of incoordination (2 males and 3 females vs 0 controls) and decreased extensor-thrust (3 each sex vs 0 controls). Dermatitis was observed in the females from Days 64-91 (3-4 treated vs 0 controls). In females (n=9-10) at Week 13, the incidence of alopecia and skin ulcer increased (20-30% treated vs 0% controls), as did the incidence of skin acanthosis, hair follicle atrophy, and ulcer (20% treated [each lesion] vs 0% controls). In males at Week 13, an increase in relative kidney weight (19%) and increased incidence of slight kidney tubular degeneration (67% treated vs 10% controls) were observed. However, these kidney effects were minimal, not dose-dependent, and were not considered to be adverse. Other microscopic lesions were increased in incidence (n=9-10) and were considered to be equivocally treatment-related as follows: (i) thyroid cysts in males (89% treated vs 40% controls); (ii) very slight to slight lung perivascular inflammation in females (50% treated vs 10% controls); and (iii) very slight to slight mesenteric tissue inflammation in females (40% treated vs 0% controls).

Similar observations occurred in the recovery group during treatment. Both sexes recovered completely from abnormal clinical observations, except one female exhibiting dermatitis at Day 119. Alopecia and skin ulcer were observed in 10% treated (each lesion) vs 0% controls. Recovery in the animals was also evident during pathology.

The LOAEL was 100 ppm (equivalent to 6.6/8.0 mg/kg/day in males/females), based on mortality in males, neuromuscular effects in both sexes, dermatitis, and gross and microscopic skin lesions in females. The NOAEL is 50 ppm (equivalent to 3.4/4.2 mg/kg/day in males/females).

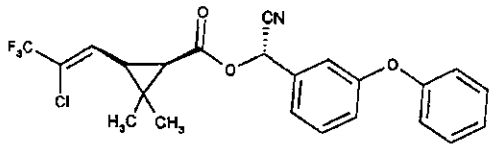
This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3100a; OECD 408) for a subchronic oral toxicity study in the rat.

COMPLIANCE - Signed and dated Data Confidentiality, GLP, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: XR-225

Description:	Off-white, odorless solid
Lot/Reference #:	CH519JMi87ABISE, TSN101800
Purity:	100% a.i.
Compound Stability:	The test material was stable in the diet for up to 14 days (temperature unspecified).
CAS # of TGAI:	76703-62-3
Structure:	

2. Vehicle - Diet

3. Test animals

Species:	Rat
Strain:	Wistar Han
Age/group mean weight at study initiation:	Approximately 7 weeks old; 192-200 g males and 136-139 g females
Source:	Charles River Laboratories Inc. (Raleigh, NC)
Housing:	Individually in suspended stainless steel cages with wire mesh floors
Diet:	Purina Certified Rodent Lab Diet #5002 meal (PMI, St. Louis, MO), *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions	
Temperature:	19-25°C
Humidity:	40-70%
Air changes:	12-15/hr
Photoperiod:	12 hrs light/12 hrs dark
Acclimation period:	14 days

B. STUDY DESIGN

1. In life dates - Start: 12/21/99 End: 04/18/00

2. Animal assignment - Animals were randomly assigned, stratified by body weight, to the test groups presented in Table 1.

Table 1. Study design. [a]

Dose (ppm)	Mean Chemical Intake (mg/kg bw/day) [M/F]	Main Study 13 Weeks (M/F)	Recovery Study 4 Weeks (M/F) [b]
0	0/0	10	10
2.5	0.2/0.2	10	0
10	0.7/0.8	10	0
50	3.4/4.2	10	0
100	6.6/8.0	10	10

a Data were obtained from MRID 45447322, pages 22, 139, and 140.
b Recovery animals were observed for 4 weeks after termination of dosing.

3. **Dose-selection rationale** - The dose levels were selected based on the results from a 4-week oral toxicity study in Wistar rats (MRID 45447321) treated with XR-225 and 90-day oral toxicity studies (*MRID or report numbers were not provided*) in Wistar rats treated with cyhalothrin or lambda cyhalothrin. The 4-week study is summarized in Appendix 1 of this DER. In the 4-week study XR-225 (gamma cyhalothrin; purity unknown; Lot/Reference #CH519-JMi-61-6) was administered to 10 Wistar Han rats/sex/group in the diet at dose levels fo 0, 2.5, 10, 50, 125, or 250 ppm (0/0, 0.2/0.2, 0.8/1.0. 4.2/4.5, 8.2/9.5, 8.8/10.2 mg/kg/day in males/females, respectively) for 4 weeks. At 125 ppm, decreased body weight//body weight gain and food consumption were observed. Clinical signs of postural and muscular abnormalities, clear perioral soiling, incoordination, decreased extensor-thrust and touch response, and increased salivation were observed. Males showed increased levels of alanine and aspartate aminotransferase, urea nitrogen and relative kidney weights. In females, total protein and albumin were decreased, and relative kidney weight was increased. Increased levels of protein and ketones were found in the urine of both sexes. The 250 ppm group was terminated on Day 5 due to moribundity. In the 90-day studies, Wistar rats were fed diets containing 0, 10, 50, or 250 ppm test compound. At 250 ppm in each study, decreased body weight, body weight gain, and food consumption were observed, as well as increased relative liver weights and liver aminopyrine-N-demethylase activity. The NOAEL was 50 ppm.

4. **Treatment preparation, administration, and analysis** - Dietary formulations were prepared weekly by serially diluting a concentrated test material-feed mixture (premix) with appropriate amounts of feed to achieve the desired test concentrations, except the 100 ppm dose was prepared by diluting the test compound with feed. The homogeneity (top, middle, bottom) of the 2.5 and 100 ppm dietary formulations was evaluated on Days -5, 35, and 74. The stability of 2.5 and 250 ppm dietary formulations after 14 days of storage (temperature not reported) was determined previously (MRID 45447321). Concentration analyses were performed Days -5, 35, and 74 for all dietary formulations, including the control.

Results

Homogeneity Analysis: Range of % coefficient of variation over both doses: 0.95-3.02%

Stability Analysis: Range of % of nominal over both doses: 97-117%

Concentration Analysis: Range as % of nominal for all groups: 93-107%

The analytical data indicated that the mixing procedure was adequate, diets were homogeneously mixed, and that the variance between nominal and actual dosage to the animals was acceptable.

5. **Statistics** - Desciptive statistics only (means and standard deviations) were reported for body weight gains, RBC indices, and WBC differential counts. All other data were subjected to the statistical procedures listed below.

Parameter	Statistical procedure
Body weights, food consumption, organ weights, clinical chemistry, enzyme, hematology, and urine specific gravity	Bartlett's test for equality of variance, parametric or nonparametric analysis of variance, followed by Dunnett's test or Wilcoxon Rank-Sum test with a Bonferroni correction as appropriate
Detailed clinical observation incidence scores	Z-test of proportions (Bruning, J.L. and Kintz, B.L.. 1977. *Computational Handbook of Statistics*. Scott, Foresman and Co., Illinois)

Outliers were identified by the Sequential Outlier test (Grubbs, F.E. 1969). Procedures for detecting outlying observation in samples. *Technometrics* 11[1]:1-21). Outliers were routinely excluded from statistical analysis of food consumption analysis, but were also excluded from others when documented, scientifically sound evidence existed for doing so.

C. METHODS

1. Observations

a. Cageside observations - Animals were checked for mortality, moribundity, and clinical signs of toxicity twice daily.

b. Clinical and neurological examinations - Examinations were performed prior to the study and weekly thereafter for all animals and included cage-side, hand-held, and open-field observations. Motor activity, rectal temperature, sensory evaluation, and grip performance evaluations were not performed. The following parameters were examined.

HOME CAGE OBSERVATIONS	HANDLING OBSERVATIONS	OPEN FIELD OBSERVATIONS
Posture	Reactivity	Responsiveness to touch
Convulsions	Lacrimation/ chromodacryorrhea	Gait abnormalities / posture
Tremors	Salivation	Gait score
Abnormal Movements	Palpebral closure	
Palpebral closure	Muscle tone	**NEUROMUSCULAR OBSERVATIONS**
Excessive soiling	Pupil size	Hindlimb extensor strength

2. **Body weight** - All animals were weighed prior to treatment, twice during the first week, and weekly thereafter during dosing and recovery periods. Prior to necropsy, a fasting body weight was measured.

3. **Food consumption and compound intake** - Food consumption (g/animal/day) was recorded prior to treatment, twice during the first week, and at least weekly thereafter during dosing and recovery periods. Compound intake (g/kg bw/day) values were calculated from the food consumption, body weight gain data, and actual feed concentration. Food efficiency was not reported.

4. **Ophthalmoscopic examination** - Ophthalmoscopic examinations were performed on Day -6 on all animals and Day 88 on animals in the main study.

5. **Hematology & clinical chemistry** - Blood was collected from the orbital sinus of all animals (fasted, duration not specified) after 4 weeks of treatment and at the scheduled sacrifices. Hematology was conducted at both time points; however, clinical chemistry was performed only at scheduled sacrifice. The following CHECKED (X) parameters were examined in the main study animals.

a. **Hematology**[a]

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc. (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

[a]For the recovery group, females only, the HCT, HGB, RBC, and red blood cell indices were analyzed.

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
	ENZYMES	X	Total protein*
X	Alkaline phosphatase (ALK)*	X	Triglycerides
	Cholinesterase (ChE)		Serum protein electrophoresis
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

In the recovery group, parameters were measured in the females only and included the following: HCT, HGB, RBC, MCH, MCV, MCHC, urea nitrogen, glucose, albumin, and calcium.

6. **Urinalysis** - Urine was collected from nonfasted animals of the main study during Week 13 and the recovery group near the end of the recovery period using timed urine volume collection and manual compression of the bladder. Microsediment was examined in the urine collection from manual compression; other urinalyses were conducted on the timed urine collection. The CHECKED (X) parameters were examined.

X	Appearance (clarity/color)*	X	Glucose
X	Volume*	X	Ketones
X	Specific gravity*	X	Bilirubin
X	pH*	X	Blood*
X	Sediment (microscopic)		Nitrate
X	Protein*	X	Urobilinogen

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

7. **Sacrifice and pathology** - All animals that died and those sacrificed on schedule were subjected to gross pathological exam, and the CHECKED (X) tissues were collected for histological examination. Additionally, the (XX) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+		GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		UROGENITAL	X	Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroid*
X	Rectum*	X	Urinary bladder*	X	Thyroids*
XX	Liver*+	XX	Testes*+		OTHER
	Gall bladder (not rat)*	XX	Epididymides*+	X	Bone and joint
	Bile duct (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicles*	X	Skin*
	RESPIRATORY	XX	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	XX	Uterus*+		
X	Lung*	X	Mammary gland*	X	Sebaceous gland
X	Nasal tissues*	X	Vagina and cervix	X	Mediastinal tissues
	Pharynx*	X	Coagulating glands	X	Mesenteric tissues
X	Larynx*	X	Oviducts	X	Oral tissues

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
\+ Organ weights required for rodent studies.

Tissues examined microscopically included all samples from the control and 100 ppm groups in the main study and all animals that died spontaneously. The liver, kidneys, lungs, and relevant gross lesions were examined in all groups of the main study. Only the liver and kidneys were weighed in the recovery group. Samples of the liver from 3 rats/sex from the control and 100 ppm groups of the main study were analyzed by transmission electron microscopy and micrographs were taken.

8. **Liver Enzymes** - Liver microsomes were prepared from all animals in the control and 100 ppm groups at both sacrifices, and enzymatic activity was measured as ethoxyresorufin, methoxyresorufin, pentoxyresorufin, O-dealkylase, ρ-nitrophenol hydroxylation, and aminopyrine-N-demethylase metabolic activities as a measure of CYP1A1, CYP1A2, CYP2B1/1, and CYP2E1 activities.

II. RESULTS

A. OBSERVATIONS

1. **Mortality** - In the 100 ppm male rats, 3/20 in the main and recovery studies died, and these deaths were considered related to treatment. These animals exhibited decreased food consumption and body weight gain; incoordination; and decreased extensor thrust response, resistance to removal, resistance to handling, responsiveness to touch, and muscle tone. One control female and one 2.5 ppm female died, and these deaths were considered incidental.

2. **Clinical signs of toxicity** - In the main study at 100 ppm, decreased moderate to pronounced extensor-thrust response was observed in both sexes throughout the study (1-3 treated each sex vs 0 controls), and this decrease was significant ($p \leq 0.05$) in the males from Days 36-71 (Table 2). Additionally, slight to pronounced incoordination was observed in 2 males and 1-3 females (vs 0 controls). Dermatitis was observed in the females from Day 64-91 (3-4 treated vs 0 controls). Similar observations occurred in the recovery group, but complete recovery was demonstrated in males and only one female still exhibited dermatitis by Day 119.

Table 2. Selected clinical observations (# rats affected/week) in main study rats treated with XR-225 for up to 13 weeks.[a]

	Dose (ppm)				
Parameter	0	2.5	10	50	100
Males					
Decreased extensor thrust response					
Moderate	0	0-1	0-1	0-1	1-3
Pronounced	0	0	0	0	0-1
Total	0	0-1	0-1	0-1	1-3
Incoordination					
Slight	0	0	0	0	1-2
Moderate	0	0	0	0	0-1
Pronounced	0	0	0	0	0-1
Total	0	0	0	0	2
Females					
Decreased extensor thrust response, Moderate	0	0	0	0-1	1-3
Incoordination, Slight	0	0	0	0	1-3
Dermatitis (Day 64-91)	0	0	0	0	3-4

a Data (n=10) were obtained from MRID 45447322 on pages 72-102. Except as noted, observations are for Days 8-91.

B. BODY WEIGHT AND BODY WEIGHT GAIN: No treatment-related effect was observed on body weight and body weight gain (Tables 3a and 3b). In the main study animals, decreased ($p \leq 0.05$) body weights were observed at 100 ppm in males on Days 4 and 8 (↓9%, each) and females on Days 4-22 and 36 (↓6-9%). A decrease in body weight gain was observed in the 50 ppm males at Day 8 (↓14%) and in the 100 ppm group on Day 8 (↓51-58%) and Day 36 (↓21-22%), which suggested a treatment-related effect. However, these decreases coincided with decreases in food consumption, and may be due to a lack of diet palatability. At 100 ppm, overall (Days 1-91) body weight gain decreased by 11% in the males and by 3% in females. Furthermore, final body weights were decreased by only 2-5% in the 100 ppm group. Similar results were observed in the recovery group with minor decreases observed in body weights at 100 ppm on Days 91 and 119 in males (↓8-10%) and females (↓3-4%). Body weight gains became more similar to controls throughout the study in males, whereas in females decreases in body weight gain were observed at Days 1-8 (↓27%), Days 1-36 (↓4%), Days 1-91 (↓8%) and Days 1-119 (↓11%).

Table 3a. Mean (±SD) body weights (g) at selected intervals in main study rats treated with XR-225 in the diet for up to 13 weeks.[a]

Days on Study	Dose (ppm)				
	0	2.5	10	50	100
Males					
1	197.6±8.4	197.2±11.0	192.3±13.7	196.7±7.7	196.2±10.5
8	233.0±10.9	233.6±14.2	232.4±12.0	226.9±10.1	211.1±17.9* (↓9)
36	308.1±26.0	321.7±27.8	317.0±25.8	314.5±23.3	284.1±24.7
91	384.5±41.3	399.5±39.3	395.1±39.5	392.1±32.5	364.9±33.7
Females					
1	137.8±5.0	137.8±5.7	139.1±7.0	136.8±5.8	135.9±6.1
4	144.5±6.0	143.7±4.9	146.6±7.6	141.6±7.9	132.0±6.9* (↓9)
22	179.0±10.8	182.0±9.7	182.6±8.3	176.9±8.6	168.6±6.1* (↓6)
91	230.6±18.4	237.1±18.2	233.5±13.6	228.7±13.5	225.7±12.0

a Data were obtained from MRID 45447322 on pages 107, 108, 112, and 113. Numbers listed parenthetically represent the percent difference from controls (calculated by reviewers).

* Significantly different from controls at $p \leq 0.05$

Table 3b. Mean (±SD) cumulative body weight gains (g) at selected intervals in main study rats treated with XR-225 in the diet for up to 13 weeks.[a]

Interval (Days)	Dose (ppm)				
	0	2.5	10	50	100
Males					
1-8	35.3±5.2	36.4±5.1	40.1±9.2	30.2±4.5 (↓14)	14.9±10.5 (↓58)
1-36	110.5±20.9	124.5±18.0	124.7±26.2	117.8±20.8	86.0±19.1 (↓22)
1-91	186.9±36.2	202.4±29.7	202.8±38.9	195.4±31.7	166.7±27.4 (↓11)
Females					
1-8	15.2±3.5	16.9±3.1	16.1±3.0	14.4±4.1 (↓5)	7.5±5.2 (↓51)
1-36	57.3±10.2	62.2±8.1	56.5±8.1	58.8±9.4	45.3±9.4 (↓21)
1-91	92.8±14.8	100.2±15.5	94.4±11.3	91.9±11.8	89.8±10.6 (↓3)

a Data were obtained from MRID 45447322 on pages 109-111 and 114-116. Numbers listed parenthetically represent the percent difference from controls (calculated by reviewers). Statistical analysis was not performed.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption - No treatment-related effect was observed on food consumption. Food consumption was decreased ($p \leq 0.05$) in both sexes at 50 ppm (↓20%, each) and 100 ppm (↓44%, each) on Days 1-4 (Table 4). At 100 ppm, food consumption was also decreased ($p \leq 0.05$) on Days 4-8, 8-15, and 31-36 in males (↓12-23%) and Days 4-36 in females (↓9-15%). Thus, the greatest decrease in food consumption occurred within the first 4 days of dosing, and food consumption was similar to the controls following Day 36. Consequently, this effect was transient and may be due to a lack of diet palatability. Food consumption in the recovery group was similar to controls in males, but incidental increases ($p \leq 0.05$) were observed in females at Days 91-99 (↑14%) and 113-119 (↑9%).

Table 4. Mean (±SD) food consumption (g/animal/day) at selected intervals in main study rats dosed with XR-225 for up to 13 weeks.[a]

Days	Dose (ppm)				
	0	2.5	10	50	100
Males					
1-4	21.0±1.2	21.2±1.4	20.6±1.8	16.7±1.9* (↓20)	11.7±2.9* (↓44)
8-15	21.9±1.5	22.9±1.9	21.9±1.8	21.9±1.2	19.2±3.5* (↓12)
85-91	20.0±1.7	20.9±1.9	20.3±2.4	19.6±2.0	19.9±2.0
Females					
1-4	14.8±0.8	14.4±1.8	15.2±1.1	11.9±1.2* (↓20)	8.3±1.4* (↓44)
22-30	16.5±1.0	16.7±1.3	16.3±1.1	16.1±1.1	15.0±1.1* (↓9)
85-91	15.3±0.9	16.0±1.2	16.2±1.1	15.7±1.2	15.7±1.3

a Data were obtained from MRID 45447322 on pages 129-132. Numbers listed parenthetically represent the percent difference from controls (calculated by the reviewers).

* Statistically different ($p \leq 0.05$) from the controls

2. Compound consumption - Compound intake values (mg/kg/day) are presented in Table 1 of this DER.

D. OPHTHALMOSCOPIC EXAMINATION - No treatment-related effect was observed during opthalmoscopic examination.

E. BLOOD ANALYSES

1. Hematology - No treatment-related effect was observed in hematology. Minor decreases ($p \leq 0.05$) were observed in erythrocytes and hemoglobin in 100 ppm females at Week 13 (↓3-5%), however, these effects were not considered to be treatment-related.

2. Clinical chemistry - No treatment-related effect was observed for clinical chemistry. Increased ($p \leq 0.05$) urea nitrogen (↑22%) and decreased ($p \leq 0.05$) albumin and glucose (↓14% and 21%, respectively) were observed in the 100 ppm females at Week 13, however, other clinical and pathological data did no corroborate an adverse effect. A minor decrease ($p \leq 0.05$) in calcium was observed in the 100 ppm females at Week 13 (↓4%). Glucose was decreased ($p \leq 0.05$) by 17% during recovery in the 100 ppm group females at Week 17. Other values in the main and recovery groups were similar to controls.

F. URINALYSIS: No treatment-related adverse effect was observed.

G. SACRIFICE AND PATHOLOGY

1. Organ weight - Increased ($p \leq 0.05$) relative to body kidney weight was observed in the 100 ppm males at Week 13 (↑19%). Slight increases ($p \leq 0.05$) in relative to body kidney (females) and liver weights (both sexes) were observed in the 100 ppm group and in the 50 ppm male relative to body liver weight (↑12-13%), but without corroborating pathological evidence of an adverse effect on the organ.

2. Gross pathology - The incidence of alopecia and skin ulcer in the 100 ppm main study females (n=9-10) was increased (20-30% treated vs 0% controls; Table 5). Following 4 weeks of recovery (n=10), alopecia and skin ulcer were observed in 10% treated (each lesion) vs 0% controls. No other gross lesion was considered treatment-related.

Table 5. Incidence (%) of selected skin and subcutis gross lesions in the main study female rats fed XR-225 in the diet for up to 13 weeks.[a]

	Dose (ppm)				
Lesion	0	2.5	10	50	100
Alopecia	0	0	0	0	30
Erosion - ulcer	0	0	0	0	20

a Data (n=9-10) were obtained from MRID 45447322 on page 180.

3. Microscopic pathology - Several microscopic lesions were increased in incidence in the 100 ppm main study group (n=9-10) as follows: (i) very slight kidney tubular degeneration in males (67% treated vs 10% controls); (ii) skin acanthosis, hair follicle atrophy, and ulcer in females (20% treated [each lesion] vs 0% controls; (iii) thyroid cysts in males (89% treated vs 40% controls); (iv) very slight to slight lung perivascular inflammation in females (50% treated vs 10% controls); and (v) very slight to slight mesenteric tissue inflammation in females (40% treated vs 0% controls; Table 6). There was no corroborating evidence of a toxic effect on the thyroid, lung, or mesenteric tissue; therefore, an effect on these organs was considered to be equivocal. No treatment-related effect was observed during electron microscopy of the liver. Microscopic pathology was not performed on the recovery study animals.

Table 6. Incidence (%) of selected non-neoplastic microscopic lesions in the main study rats fed XR-225 in the diet for up to 13 weeks. [a]

	Dose (ppm)				
Microscopic lesion	0	2.5	10	50	100
Males					
Kidneys, tubular degeneration, very slight	10	20	10	20	67
Thyroid, cyst, total	40	—	—	—	89
Females					
Skin, acanthosis, total	0	—	0[b]	—	20
hair follicle atrophy	0	—	0[b]	—	20
ulcer	0	—	100[b]	—	20
Lung, perivascular inflammation, total	10	11	20	10	50
very slight	10	11	20	10	40
slight	0	0	0	0	10
Mesenteric tissue, inflammation, total	0	—	0[b]	—	40
very slight	0	—	0[b]	—	10
slight	0	—	0[b]	—	30

a Data (n=9-10) were obtained from MRID 45447322 on pages 202, 204, 205, 208, 209.
b Only one animal examined
— Not examined

H. LIVER ENZYMES: *p*-Nitrophenol hydroxylation was decreased ($p \leq 0.05$) by 55% in the 100 ppm females; however, other enzyme activities were similar to controls, and there was no other clinical or gross or microscopic pathological evidence of hepatotoxicity. The decrease in *p*-nitrophenol hydroxylation may have been incidental or an adaptive response.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS: The LOAEL was 100 ppm, based on mortality in males; neuromuscular signs in both sexes; dermatitis and gross and microscopic skin lesions in females.

B. REVIEWER'S COMMENTS:

No treatment-related adverse effects were observed on body weight/body weight gain, food consumption, opthalmology, hematology, or mixed function oxidase activity at 2.5, 10, or 50 ppm. Minor fluctuations (decrease) in body weight/body weight gains were observed at 100 ppm but were not considered treatment-related.

At 100 ppm, target organs seem to be skin and neuromuscular system. Similar toxicity effects were observed in a 4-week range-finding study. Mortality in high dose males (3/20) was considered to be treatment-related. At 100 ppm, animals showed clinical signs of incoordination (2 males and 3 females vs 0 controls) and decreased extensor-thrust (3 each sex treated vs 0 controls). At 100 ppm, dermatitis was observed in the females from Days 64-91 (3-4 treated vs 0 controls). In females (n=9-10) at Week 13, the incidence of alopecia and skin ulcer increased (20-30% treated vs 0% controls), as did the incidence of skin acanthosis, hair follicle atrophy, and ulcer (20% treated [each lesion] vs 0% controls). In males at Week 13, increase in relative kidney weighs (19%), and increased incidence of slight kidney tubular degeneration (67% treated vs 10% controls) were observed. However, these kidney effects were minimal, not dose-dependent, and not considered to be adverse. Other microscopic lesions were increased in incidence (n=9-10) and were considered to be equivocally treatment-related as follows: (i) thyroid cysts in males (89% treated vs 40% controls); (ii) very slight to slight lung perivascular inflammation in females (50% treated vs 10% controls); and (iii) very slight to slight mesenteric tissue inflammation in females (40% treated vs 0% controls).

Similar observations occurred in the recovery group during treatment. Both sexes recovered completely from abnormal clinical observations, except one female exhibiting dermatitis on Day 119. Alopecia and skin ulcer were observed in 10% treated (each lesion) vs 0% controls. Recovery in the animals was also evident during pathology.

The LOAEL was 100 ppm (6.6/8.0 mg/kg/day in males/females), based on mortality in males, neuromuscular effects in both sexes, dermatitis, and gross and microscopic skin lesions in females.

C. STUDY DEFICIENCIES: Complete summary data for male hematology was not provided; this was considered to be a minor deficiency that does not change the conclusions of this review.

DATA FOR ENTRY INTO ISIS

Subchronic Oral Study - rodents (870.3100); XR-225 (gamma cyhalothrin)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
128867	45447322; 45447321	subchronic	rat	13 weeks	oral	diet	0.2-8.0	0/0, 0.2/0.2, 0.7/0.8, 3.4/4.2, 6.6/8.0 (M/F)	3.4	6.6	mortality, skin, kidney, neuromus-cular system	

APPENDIX 1

Reference: Johnson, K.A. T.K. Jeffries, S.J. Day, *et al.* (1999) XR-225: 4-week repeated dose dietary toxicity study in Wistar Han rats. Toxicology & Environmental Research and Consulting, Midland, MI. Laboratory Project Study Id.: 991089, October 6, 1999. MRID 45447321. Unpublished.

Since this range-finding study was performed to determine adequate dose levels for subsequent studies, only a summary is provided.

In a range-finding oral toxicity study (MRID 45447321), XR-225 (gamma cyhalothrin; purity not reported; Lot/Reference # 519-JMi-61-6) was administered to 10 Wistar Han rats/sex/group in the diet at dose levels of 0, 2.5, 10, 50, 125, or 250 ppm (equivalent to 0/0, 0.2/0.2, 0.8/1.0, 4.2/4.5, 8.2/9.5, 8.8/10.2 mg/kg/day in males/females) for 4 weeks.

No treatment-related adverse effects were observed on ophthalmology, prothrombin time, mixed function oxidase activity, or microscopic pathology. No treatment-related effects were observed at 2.5, 10, or 50 ppm.

At 125 ppm, body weights were decreased ($p<=0.05$) by 6-18% in males throughout the study and by 9-10% on Days 4-15 in females. Overall (Days 1-29) body weight gain was decreased by 24-45% in both sexes. Food consumption was decreased ($p<=0.05$) by 23-57% in males and 12-51% in females throughout the study, but became more similar to controls in both sexes with time. Numerous clinical signs of toxicity were observed as follows (vs 0/10 controls): (i) postural abnormalities (arched back, hindlimbs splayed, walks on toes, and/or knuckling) on Days 4 and 5 (3-10/10 each sex); (ii) clear perioral soiling at Days 4 and 5 (2-4/10, each sex); (iii) slight to moderate incoordination at Days 4 and 5 (2-10/10, each sex); and (iv) abnormally soft/limp muscles at Day 5 (2-6/10, each sex). Additionally, moderate decreased extensor-thrust response and responsiveness to touch was observed in males at Days 22-29 (1-3/10 treated, each lesion vs 0/10 controls) and increased moderate salivation was observed in females at Day 29 (3/10 treated vs 0/10 controls). In males at Day 29, serum alanine and aspartate aminotransferase were increased ($p<=0.05$) by 27% and 20%, respectively; however, pathology did not corroborate hepatotoxicity. At 125 ppm in the males, serum urea nitrogen was increased ($p<=0.05$) by 14% and relative to body kidney weights were increased ($p<=0.05$) by 11%. In females, total protein and albumin were decreased ($p<=0.05$) by 12-14%, and relative to body kidney weight was increased ($p<=0.05$) by 6%. Increased levels of protein and ketones were found in the urine of both sexes.

The 250 ppm group was terminated on Day 5 due to moribundity evidenced as postural abnormalities (hindlimbs splayed, walks on toes, and knuckling), incoordination, piloerection, soiling; food consumption decreased ($p<=0.05$) by 66-71%; and body weights decreased ($p<=0.05$) by 17-19% at Day 4, and the animals lost 15-22 g during the Day 1-4 interval.

H

DATA EVALUATION RECORD

XR-225 (GAMMA CYHALOTHRIN)

Study Type: §83-3a; Developmental Toxicity Study in Rats

Work Assignment No. 1-01-03 C and D; Formerly 5-01-215 C and D
(MRIDs 45447324 and 45447323)

Prepared for
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Template version 11/01

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DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study - Rat; OPPTS 870.3700a [§83-3a]; OECD 414.

PC CODE: 128807

DP BARCODE: D288053, D288062, D288067
SUBMISSION NO.: S625041, S628578, S628592

TEST MATERIAL (PURITY): XR-225 (Gamma cyhalothrin; 100.0% a.i.)

SYNONYMS: Cyclopropanecarboxylic acid, 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl-, cyano(3-phenoxyphenyl)methyl ester, [1R-[1.alpha(S*),3.alpha.(Z)]]; X670225

CITATIONS: Marty, M. S., and Zablotny, C. L. (2000) XR-225: oral gavage developmental toxicity study in CD rats. Dow Chemical Company, Toxicology and Environmental Research and Consulting, Midland, MI. Laboratory Project Study ID No.: 991250, July 13, 2000. MRID 45447324. Unpublished.

Marty, M. S., Zablotny, C. L., and Johnson, K. A. (2000) XR-225: oral gavage developmental toxicity probe study in CD rats. Dow Chemical Company, Toxicology and Environmental Research and Consulting, Midland, MI. Laboratory Project Study ID No.: 991192, January 20, 2000. MRID 45447323. Unpublished.

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Rd., Indianapolis, IN

EXECUTIVE SUMMARY: In a primary developmental toxicity study (MRIDs 45447324), XR-225, (Gamma cyhalothrin; 100.0% a.i.; Lot/Batch # CH519MI87ABISE, TSN101800) in a corn oil vehicle, was administered daily by oral gavage at a dose volume of 4 mL/kg bw to 25 pregnant female CD rats/group at dose levels of 0, 0.1, 0.5, or 2.0 mg/kg on gestation days (GD) 6 through 20. All dams were sacrificed on GD 21; their fetuses were removed by cesarean and examined. The dose levels selected for this primary study were chosen based on the results of a developmental toxicity range-finding study (MRID 45447323).

In the primary developmental study, at 2.0 mg/kg, one animal exhibited multiple signs of toxicity on GD 21, including decreased activity, red vulvar discharge, red perioral staining, and red body soiling. Clear perioral soiling was observed in 16/25 dams on multiple occasions. Decreased feces w observed in 3/25 dams, with abdominal urine soiling also noted in one of these animals. A partially closed eye lid was noted on 4 days in a single animal. Significant treatment-related effects were observed in maternal body weights and food consumption. Body weight was decreased by 7% on GD 21; body weight gain was decreased by 16-53% on GDs 6-9, 18-21, throughout treatment when not corrected for gravid uterine weights (GD 6-21), when corrected for gravid uterine weights (statistics not performed), and for the overall (GD 0-21) study. Gravid uterine weights were comparable to controls. Terminal body weights were decreased by 7% in the 2.0 mg/kg dams. Food consumption was decreased by 12-20% in the 2.0 mg/kg dams on GD 6-12 and 18-21.

The maternal LOAEL is 2.0 mg/kg/day, based on decreased body weight, body weight gain, and food consumption, and clinical signs of toxicity. The maternal NOAEL is 0.5 mg/kg/day.

No effects of treatment were noted on numbers of litters, live fetuses, early resorptions, or on sex ratio or post-implantation losses. There were no abortions, premature deliveries, complete litter resorptions, or dead fetuses. Late resorptions were observed at 2.0 mg/kg (12 treated vs 0 controls); however, 11 of these occurred in one litter. There were no treatment-related alterations in fetal growth. There were no treatment-related external, visceral, or skeletal malformations or variations.

The developmental LOAEL was not observed. The developmental NOAEL is 2.0 mg/kg/day.

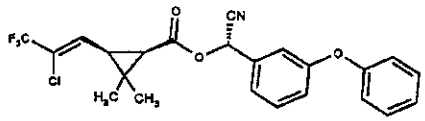
This study is classified **acceptable/guideline (OPPTS 870.3700a)** and satisfies the requirements for a developmental study in the rat.

COMPLIANCE: Signed and dated Data Confidentiality, GLP, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test material:** XR-225 (Gamma cyhalothrin)

Description:	Off-white solid
Lot/Batch #:	CH519MI87ABISE, TSN101800
Purity:	100.0% a.i.
Compound Stability:	Low and mid dose suspensions stable for 28 days (temperature not provided) High dose suspension stable for 22 days (temperature not provided)
CAS # of TGAI:	76703-62-3
Structure:	

2. **Vehicle and/or positive control:** Corn oil

3. **Test animals:**

Species:	Rat	
Strain:	CD (CRL)	
Age/group mean weight on GD 0:	Approximately 10-11 weeks/200.0-225.4 g	
Source:	Charles River Laboratories, Portage, MI	
Housing:	Individually in suspended stainless steel cages with wire mesh floors	
Diet:	Purina Certified Rodent Lab Diet 5002 (Purina Mills, Inc., St. Louis, MO), *ad libitum*	
Water:	Municipal water, *ad libitum*	
Environmental conditions:	**Temperature:**	19-25°C
	Humidity:	40-70%
	Air changes:	12-15/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	4-5 days	

B. PROCEDURES AND STUDY DESIGN

1. **In life dates** - Start: January 7, 2000 End: February 15, 2000

2. **Mating:** Sexually mature, nulliparous females were paired overnight with males of the same strain (1 male : 1 female) at Charles River Laboratories. Confirmation of mating was determined

by the presence of a copulation plug, and the day on which mating was confirmed was designated as gestation day (GD) 0.

3. **Animal assignment:** After arrival, dams were stratified by body weight and randomly assigned to dose groups as indicated in Table 1.

Table 1. Animal assignment [a]

Dose (mg/kg bw/day)	0	0.1	0.5	2.0
# Females	25	25	25	25

a Obtained from the study report (MRID 45447324) on page 16

4. **Dose selection rationale:** The dose levels summarized in Table 1 were chosen based on the results of a developmental toxicity range finding study (MRID 45447323). In the range-finding study, cyhalothrin (100% a.i.) was administered at the levels of 1, 2.5, 5, and 15 mg/kg/day to 8 pregnant female CD rats/group on gestation days 6-20. Excessive maternal toxicity was observed including severe neurological symptoms at doses ≥5 mg/kg/day. At 1 mg/kg/day, animals showed decreased body weights by 22% generally throughout treatment. At 2.5 mg/kg/day, incoordinated gait (2/8 dams), clear perioral stain (7/8 animals), and clear or red periocular stain (1/8 animals) were observed. Significant decreases in body weight (7%) on GD 21 and in body weight gain (19-35%) were observed on GD 15-21, GD 6-21, and GD 0-21. Terminal body weights were significantly decreased (7%). In this range finding study, the maternal NOAEL was not observed and LOAEL was 1.0 mg/kg/day, based on decreased body weight gain. No other effects of treatment were noted. The developmental NOAEL was 2.5 mg/kg/day; the developmental LOAEL was not observed. A summary is included as an Appendix at the end of this DER.

5. **Dosage preparation and analysis:** Dosing solutions were prepared prior to the first day of administration and periodically throughout the dosing period by suspending a weighed amount of test substance in corn oil. Storage conditions for the dose mixtures were not provided. Homogeneity of the test substance in the vehicle was determined concurrent with the start of the study for the first 0.1 and 2.0 mg/kg bw/day formulations. Stability of the test substance in the vehicle from the first 0.1 and 0.5 mg/kg bw/day mixtures was determined for 28 days (temperature not provided); stability of the first 2.0 mg/kg bw/day mixture was determined for 22 days following remixing (temperature not provided). The concentration of the test substance at each dose level was determined on the first and final dose mixtures. Additionally, the initial high dose formulation was remixed and reanalyzed due to a lower than target initial concentration.

Results -

Homogeneity (% RSD):

0.1 mg/kg bw/day	2.02%
2.0 mg/kg bw/day	5.76%

Test material was homogeneously mixed.

Stability - (range as % initial concentration): 97.9-100%

Mean Concentration (% of target concentration):

94.4% (0.1 mg/kg/day)
93.7% (0.5 mg/kg/day)
88.8% (2 mg/kg/day)

The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the study animals was acceptable.

6. Dosage administration: All doses were administered once daily by oral gavage, on GDs 6-20, in a volume of 4 mL/kg of body weight. Dosing was adjusted daily based on individual body weights.

C. OBSERVATIONS

1. Maternal observations and evaluations: All dams were checked daily for mortality and clinical signs of toxicity. Body weights were determined on GD 0 by Charles River Laboratories, and on GD 6-21 by the performing laboratory. Food consumption (g/dam/day) was measured for GDs 3-6, 6-9, 9-12, 12-15, 15-18, and 18-21. On GD 21, all dams were sacrificed and subjected to necropsy. The liver, kidneys, and gravid uterus were weighed. Representative sections of liver, kidneys, and any gross lesions were preserved in 10% neutral phosphate-buffered formalin; however, these samples were not microscopically examined. All fetuses were removed by caesarean section. The numbers of corpora lutea, implantations, live fetuses, dead fetuses, and resorptions (early and late) were recorded.

2. Fetal evaluations: All fetuses delivered by cesarean were individually identified, sexed, weighed, and examined for external abnormalities. At least 50% of the fetuses in each litter were chosen randomly via computer for visceral examination. Additionally, the heads of these fetuses were removed, placed in Bouin's fixative, and sectioned for examination of the eyes, brain, nasal passages, and tongue by Wilson's method. The remaining fetuses were eviscerated, skinned, fixed in alcohol, double stained with alcian blue and alizarin red S for skeletal examination, and cleared in glycerin.

D. DATA ANALYSIS

1. Statistical analyses: Data were subjected to the following statistical procedures:

Parameter	Statistical test
Maternal and fetal body weights, maternal body weight gains, organ weights (absolute and relative to body), and food consumption	Bartlett's test, followed by a parametric or non-parametric ANOVA as appropriate. If significant ANOVA was observed, Dunnett's Test (two-sided) or Wilcoxon Rank-Sum test (two-sided) with Bonferroni's correction was performed as appropriate.
Frequency of pre-implantation loss, resorptions per litter, resorptions per fetal population, and fetal alterations	Censored Wilcoxon test (two-sided) with Bonferroni's correction
Number of corpora lutea and implantations, and litter size	Non-parametric ANOVA followed by Wilcoxon Rank-Sum test (two-sided) with Bonferroni's correction
Pregnancy rates	Fisher exact test with Bonferroni's correction (two-sided)
Fetal sex ratios	Binomial distribution test (two-sided)
Statistical outliers	Sequential outliers test (two-sided)

Significance was denoted at $p \leq 0.05$ for all tests except Bartlett's ($p \leq 0.01$). Because numerous measurements were compared in the same group of animals, the overall false positive (Type I error) rate was greater than the nominal α levels. Therefore, final interpretation of the data considered statistical analyses along with other factors, including dose-response relationships and consistency with other biological and pathological findings and historical control data.

2. Indices: The following indices were calculated:

% pregnant = number of females with visible implantations/ number of females bred x 100

Pre-implantation loss (%) = (number of corpora lutea - number of implantations)/number of corpora lutea x 100

3. Historical control data: Historical control data were provided for maternal terminal body weights, absolute and relative (to body) liver weights, percent implantations resorbed, and incidences of extra first lumbar ribs. Data were comprised of 6 studies performed from 1999-2000, except for the fetal skeletal variations (single study dated 10/1992).

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical observations: There were no unscheduled deaths. At 2.0 mg/kg, one animal exhibited multiple signs of toxicity on GD 21, including decreased activity, red vulvar discharge, red perioral staining, and red body soiling. Clear perioral soiling was observed in 16/25 dams on multiple occasions. Decreased feces was observed in 3/25 dams, with abdominal urine soiling also noted in one of these animals. A partially closed eye lid was noted on 4 days in a single animal. No other clinical signs of toxicity were noted.

Table 2. Maternal clinical signs [number of animals affected[a]]

Clinical sign	Dose (mg/kg/day)			
	0	0.1	0.5	2.0
Clear perioral staining	0	0	0	16
Abdominal urine soiling	0	0	0	1
Decreased activity	0	0	0	1
Decreased feces	0	0	0	3
Extensive red body soiling	0	0	0	1
Red perioral soiling	0	0	0	1
Red vulvar discharge	0	0	0	1
Right eyelid partially closed	0	0	0	1

a Data obtained from Table 4, pages 35-36, and Appendix 1, pages 47-56, of the study report (MRID 45447324).

2. Body weight: Body weight and body weight gain data are summarized in Tables 3a and b. At 2.0 mg/kg, body weight was decreased (↓7%; $p \leq 0.05$) on GD 21. Body weight gains were decreased ($p \leq 0.05$) on GDs 6-9 (↓46%), 18-21 (↓32%), throughout treatment when not corrected for gravid uterine weight (GD 6-21; ↓21%) and when corrected for gravid uterine weights (statistics not performed; ↓53%), resulting in decreased (↓16%) body weight gains for the overall (GD 0-21) study. Gravid uterine weights were comparable to controls. No treatment-related effects on body weight or body weight gains were observed at 0.1 or 0.5 mg/kg.

Table 3a. Mean (±SD) maternal body weight (g)[a]

Gestational Day	Dose in mg/kg bw/day			
	0	0.1	0.5	2.0
Pretreatment:				
Day 0	225.4±12.0	220.0±11.0	221.7±9.7	223.9±11.4
Treatment:				
Day 6	256.5±11.3	250.5±13.1	251.6±11.6	256.1±13.7
Day 9	271.2±13.3	265.7±14.0	265.4±13.1	264.2±15.3
Day 18	345.6±21.4	337.0±19.2	335.5±18.5	332.1±20.3
Post-treatment:				
Day 21	391.2±30.0	378.5±23.3	376.4±22.0	363.0±27.5* (↓7)
Day 21 (corrected)[b]	296.9±20.6	286.0±19.8	283.9±16.0	274.9±24.6* (↓7)

a Data obtained from Table 5, page 37, of the study report (MRID 45447324). Percent difference from controls, calculated by the reviewers, is included in parentheses. n=25

b Day 21 corrected weight = terminal body weight - gravid uterus weight

* Significantly different from controls, $p \leq 0.05$

Table 3b. Mean (±SD) maternal body weight gain (g)[a]

Interval		Dose in mg/kg bw/day			
		0	0.1	0.5	2.0
Pretreatment:	**Days 0-6**	31.1±4.8	30.6±6.4	29.9±6.4	32.2±5.1
Treatment:	**Days 6-9**	14.7±4.6	15.2±4.5	13.8±5.9	8.0±5.0* (↓46)
	Days 9-12	19.7±5.9	18.5±5.8	18.2±4.8	18.4±6.8
	Days 12-15	21.5±7.1	20.4±5.9	19.5±6.0	20.5±5.7
	Days 15-18	33.2±7.2	32.4±6.0	32.5±6.7	29.0±6.1
	Days 18-21	45.5±10.9	41.5±7.5	40.9±6.9	31.0±12.9* (↓32)
Overall study	**Days 0-21**	165.8±23.9	158.5±19.1	154.7±19.4	131.9±19.9* (↓16)
Days 6-21 (uncorrected)		134.7±22.9	128.0±15.4	124.9±16.1	106.9±17.2* (↓21)
Gravid uterus		94.25±22.77	92.44±12.69	92.57±15.07	88.12±15.15
Days 6-20 (corrected)[b]		40.4±13.1	35.5±13.2	32.3±12.5	18.8±18.2 (↓53)

a Data obtained from Table 6, page 38, and Table 10, page 44 of the study report (MRID 45447324). Percent difference from controls, calculated by the reviewers, is included in parentheses. n=25

b Calculated by reviewers from data in Appendix 2, pages 57-60, of the study report (MRID 45447324). Percent difference from controls is included in parentheses.

* Significantly different from controls, $p \leq 0.05$

3. **Food consumption:** Food consumption was decreased (↓ 12-20%; $p \leq 0.05$) in the 2.0 mg/kg dams on GD 6-12 and 18-21 (Table 4). Additionally, food consumption was decreased ($p \leq 0.05$) in the 0.5 mg/kg dams on GD 9-12 (↓ 8%); however, this observation was isolated, minor, and did not affect body weight or body weight gain. Food consumption was comparable to controls in the 0.1 mg/kg dams.

Table 4. Mean (±SD) maternal food consumption (g/kg/day)[a]

Interval		Dose in mg/kg bw/day			
		0	0.1	0.5	2.0
Pretreatment:	Days 3-6	21.5±1.3	20.9±1.7	20.8±1.9	21.2±1.5
Treatment:	Days 6-9	20.5±1.5	20.0±1.8	19.4±2.4	16.5±1.8* (↓20)
	Days 9-12	22.3±1.9	21.2±2.2	20.6±2.3* (↓8)	19.6±2.2* (↓12)
	Days 18-21	24.0±2.3	22.5±2.3	22.7±2.5	20.7±3.0* (↓14)

a Data obtained from Table 7, page 39, of the study report (MRID 45447324). Percent difference from controls, calculated by the reviewers, is included in parentheses. n=25

* Significantly different from controls, $p \leq 0.05$

4. **Organ weight:** Organ weight data are summarized in Table 5. Terminal body weights were decreased (↓7%; $p \leq 0.05$) in the 2.0 mg/kg dams. At ≥0.5 mg/kg, dams demonstrated decreased ($p \leq 0.05$) absolute liver weights (↓9-10%); however, relative (to body) liver weights were similar to controls. Therefore, it was concluded that the decreased absolute liver weights were due to the decreased body weights and were not adverse.

Table 5. Mean (±SD) maternal liver weights[a]

Observation	Dose in mg/kg bw/day			
	0	0.1	0.5	2.0
Terminal body weight (g)	389.5±29.7	382.0±21.6	378.9±22.0	361.6±28.7* (↓7)
Absolute liver	15.047±1.632	14.136±1.816	13.698±1.373* (↓9)	13.608±1.791* (↓10)
Relative (to body) liver	3.872±0.401	3.700±0.424	3.626±0.307	3.759±0.379

a Data obtained from Table 8, page 40, of the study report (MRID 45447324). Percent difference from controls, calculated by the reviewers, is included in parentheses. n=21-22

* Significantly different from controls, $p \leq 0.05$

5. **Gross pathology:** There were no treatment-related macroscopic findings in any group.

6. **Cesarean section data:** Cesarean section data are presented in Table 6. Late resorptions were observed in the 2.0 mg/kg group (12 vs 0 controls); however, 11 of these occurred in one litter. There were no abortions, premature deliveries, complete litter resorptions, or dead fetuses. No effects of treatment were noted on numbers of litters, live fetuses, early resorptions, or on sex ratio or post-implantation losses.

Table 6. Cesarean section observations[a]

Observation	Dose (mg/kg bw/day)			
	0	0.1	0.5	2.0
# Animals Assigned (Mated)	25	25	25	25
# Animals Pregnant	25	25	25	25
Pregnancy Rate (%)[b]	100.0	100.0	100.0	100.0
# Nonpregnant[b]	0	0	0	0
Maternal Wastage				
# Died	0	0	0	0
# Died Pregnant	0	0	0	0
# Died Nonpregnant	0	0	0	0
# Aborted	0	0	0	0
# Premature Delivery	0	0	0	0
Total # Corpora Lutea	322	322	324	322
Corpora Lutea/Dam	12.9±2.9	12.9±2.0	13.0±1.9	12.9±2.2
Total # Implantations	311	306	312	305
(Implantations/Dam)	12.4±3.2	12.2±2.2	12.5±2.2	12.2±2.6
Total # Litters	25	25	25	25
Total # Live Fetuses	302	295	296	283
(Live Fetuses/Dam)	12.1±3.1	11.8±1.9	11.8±2.1	11.3±3.4
Total # Dead Fetuses[c]	0	0	0	0
(Dead Fetuses/Dam)[c]	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Total # Resorptions	9	11	16	22
Early[c]	9	11	16	10
Late[c]	0	0	0	12
Resorptions/Dam	0.4±0.7	0.4±0.7	0.6±1.0	0.9±2.2
Early[c]	0.4±0.7	0.7±0.7	0.6±1.0	0.4±0.6
Late[c]	0.0±0.0	0.0±0.0	0.0±0.0	0.5±2.2
Litters with Complete Resorptions	0	0	0	0
Mean Fetal Weight (g)	5.68±0.24	5.73±0.28	5.72±0.27	5.61±0.42
Males	5.82±0.33	5.88±0.31	5.85±0.26	5.70±0.43
Females	5.53±0.25	5.58±0.29	5.54±0.42	5.46±0.41
Sex Ratio (% Male)	49	51	52	50
Preimplantation Loss (%)	4.7±12.0	5.1±6.1	4.1±5.7	5.5±12.3
Postimplantation Loss (%)	2.9	3.6	5.1	7.2

a Data obtained from pages 44, 73-76, and 277-449 of the study report (MRID 45447324). Percent difference from controls, calculated by reviewers, is included in parentheses.

b Calculated by reviewers

c Calculated by reviewers from individual data presented in Appendix Table 8, pages 277-449 of the study report (MRID 45447324).

B. DEVELOPMENTAL TOXICITY

1. **External examination** - External malformations are presented in Table 7a. Cutis laxis, an excessive looseness of the skin, was observed at 0.1 mg/kg (1.7% fetuses; 4.0% litters), compared to 0 controls. The Sponsor stated that this malformation had been observed in several other developmental toxicity studies on unrelated compounds (data not provided), and was considered a heritable anomaly. This conclusion was supported by the finding that all of the fetuses affected were from the same litter. This finding also was not dose-dependent; therefore, the reviewers agree with Sponsor that this finding is not treatment-related. Bilateral hindlimb rotation was observed in a single fetus at 2.0 mg/kg (0.4% fetuses; 4.0% litters), and digit polydactyly (attached to the 3rd phalange bilaterally) was observed at 0.5 mg/kg (0.3% fetuses; 4.0% litters), both compared to 0 controls. Historical control data was not provided for these anomalies. However, these findings were considered to be incidental and/or not dose-dependent, and thus not treatment-related. No other external malformations or variations were observed.

2. **Visceral examination** - Visceral abnormalities are presented in Table 7b. A single 2.0 mg/kg fetus was found to be missing its thymus (0.7% fetuses; 4.0% litters), and a single 0.1 mg/kg fetus was observed to have dilated cerebral ventricles (0.7% fetuses; 4.0% litters), compared to 0 controls. These findings were considered to be incidental and/or not dose-dependent, and thus not treatment-related. No other visceral malformations were noted. Stomach hemorrhage, a variation, was noted in one 2.0 mg/kg fetus (0.7% fetuses; 4.0% litters), intestine hemorrhage and liver hemorrhage (variations) were each found in one 0.5 mg/kg fetus (0.6% fetuses; 4.0% litters), and pale liver, a variation, was observed in a 0.1 mg/kg fetus (0.7% fetuses; 4.0% litters). Liver hemorrhage also was noted in 1 control. Historical control data was not provided for these anomalies. However, these findings were considered to be incidental and/or not dose-dependent, and were not considered treatment-related. No other visceral variations were noted.

3. **Skeletal examination** - Skeletal findings are presented in Table 7c. There were no dose-related skeletal malformations. Extra 1st lumbar ribs were observed in one fetus at 0.1 (0.7% fetuses; 4.0% litters) and 0.5 (0.7% fetuses; 4.0% litters) mg/kg, as well as in 1 control (0.7% fetuses; 4.0% litters); however, these findings fell below the incidence in the historical control study (1.1% fetuses; 8.0% litters) and were not considered treatment-related. Extra 1st lumbar ribs also were observed in 4 fetuses at 2.0 mg/kg (2.9% fetuses; 8.3% litters); however, this result was not statistically significant, 3 of the 4 fetuses came from a single litter, and the litter incidence is comparable to the historical control litter incidence (8.0%). Therefore, this finding is considered to be incidental and not compound-related. Delayed ossification of the thoracic centra was observed in the 0.1 (0.7% fetuses; 4.0% litters) and 0.5 (1.4% fetuses; 8.0% litters) mg/kg groups; however, this retardation was noted in the concurrent controls (4.1% fetuses; 16.0% litters). Delayed ossification of the sternebrae was observed at 0.1 (2.8% fetuses; 16.0% litters), 0.5 (1.4% fetuses; 8.0% litters), and 2.0 (1.5% fetuses; 8.3% litters) mg/kg; however this retardation also was noted in the concurrent controls (2.8% fetuses; 12.0% litters). Therefore, these skeletal retardations were not considered to be related to treatment.

Table 7a. External malformations [% fetuses affected (% litters affected)][a]

Observations		Dose (mg/kg bw/day)			
		0	0.1	0.5	2.0
#Fetuses (litters) examined		302 (25)	295 (25)	296 (25)	283 (25)
Digit	polydactyly	0 (0)	0 (0)	0.3 (4.0)	0 (0)
Hindlimb	rotation, bilateral	0 (0)	0 (0)	0 (0)	0.4 (4.0)
Skin	cutis laxis	0 (0)	1.7 (4.0)	0 (0)	0 (0)
Eye	microphthalmia	0.6 (4.0)	0 (0)	0 (0)	0 (0)

a Data were obtained from Table 11, page 45, in the study report (MRID 45447324).

Table 7b. Visceral abnormalities [% fetuses affected (% litters affected)][a]

Observations		Dose (mg/kg bw/day)			
		0	0.1	0.5	2.0
# of Fetuses(litters) examined		157 (25)	153 (25)	154 (25)	147 (25)
Malformations					
Thymus	missing	0 (0)	0 (0)	0 (0)	0.7 (4.0)
Brain	dilated cerebral ventricles	0 (0)	0.7 (4.0)	0 (0)	0 (0)
Variations					
Liver	hemorrhage	0.6 (4.0)	0 (0)	0.6 (4.0)	0 (0)
	pale	0 (0)	0.7 (4.0)	0 (0)	0 (0)
Stomach	hemorrhage	0 (0)	0 (0)	0 (0)	0.7 (4.0)
Intestine	hemorrhage	0 (0)	0 (0)	0.6 (4.0)	0 (0)

a Data were obtained from Table 11, page 45, in the study report (MRID 45447324).

Table 7c. Skeletal findings [% fetuses affected (% litters affected)][a]

Observations		Dose (mg/kg bw/day)				
		0	0.1	0.5	2.0	Historical Controls
#Fetuses (litters) examined		145 (25)	142 (25)	142 (25)	136 (24)	190 (25)
Variations						
Ribs	extra 1st lumbar	0.7 (4.0)	0.7 (4.0)[b]	0.7 (4.0)[b]	2.9 (8.3)	1.1 (8.0)
Retardations						
Thoracic centra	delayed ossification	4.1 (16.0)	0.7 (4.0)	1.4 (8.0)	0 (0)	NP
Sternebrae	delayed ossification	2.9 (12.0)	2.9 (16.0)	1.4 (8.0)	1.5 (8.3)	NP

a Data were obtained from Table 11, page 46, and Appendix Table 8, pages 350 and 383, in the study report (MRID 45447324).

b Data tabulated in summary Table 4, page 46 and Text Table 8, page 27 are incorrect. Individual data in Appendix Table 8, pages 350 and 383, indicate 1 fetus was affected in the 0.1 and 0.5 mg/kg dose groups.

NP Not provided

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The maternal LOAEL was 2.0 mg/kg, based on clinical signs of toxicity, lower body weight and body weight gains, and decreased food consumption. No test compound-related maternal findings were noted in the dams of the 0.1 or 0.5 mg/kg dose groups. No evidence of embryonal or fetal toxicity was observed at any dose, and there was no evidence of teratogenicity.

B. REVIEWER COMMENTS

Dose Rationale

The dose selection rationale of this primary study was based on a developmental range-finding study. In the range-finding study, cyhalothrin caused excessive maternal toxicity including severe neurological symptoms at doses ≥5 mg/kg/day. Animals were sacrificed prior to the study conclusion (GD 7-8). At 1 mg/kg/day, animals showed decreased body weights (↓22%) throughout treatment. At 2.5 mg/kg/day, incoordinated gait (2/8 dams), clear perioral stain (7/8 animals), and clear or red periocular stain (1/8 animals) were observed. Body weight was decreased (7%) on GD 21. Body weight gains were decreased (19-35%) on GD 15-21, GD 6-21, and GD 0-21. Terminal body weights were also decreased (7%). In this range-finding study, the maternal NOAEL was not observed and the LOAEL was 1.0 mg/kg/day, based on decreased body weight gains. No other effects of treatment were noted. The developmental LOAEL was not observed and the NOAEL was 2.5 mg/kg/day.

1. Maternal toxicity: There were no unscheduled deaths in the primary study. At 2.0 mg/kg, one animal exhibited multiple signs of toxicity on GD 21, including decreased activity, red vulvar discharge, red perioral staining, and red body soiling. Clear perioral soiling was observed in 16/25 dams on multiple occasions. Decreased feces were observed in 3/25 dams, with abdominal urine soiling also noted in one of these animals. A partially closed eye lid was noted on 4 days in a single animal.

At 2.0 mg/kg, significant decreases in body weight (7%) on GD 21 and body weight gain (16-53%) on GDs 6-9, 18-21 were observed throughout treatment. Body weight gain was also decreased when not corrected for gravid uterine weight (GD 6-21). When corrected for gravid uterine weights (statistics not performed), decreased body weight gain was seen for the overall (GD 0-21) study. Terminal body weights were decreased (7%; $p \leq 0.05$). Food consumption was decreased (12-20%; $p \leq 0.05$) on GD 6-12 and 18-21.

The maternal LOAEL is 2.0 mg/kg/day, based on decreased body weight, body weight gain, and food consumption, and clinical signs of toxicity (e.g.: clear perioral staining.) The maternal NOAEL is 0.5 mg/kg/day.

2. Developmental toxicity:

a. Deaths/Resorptions: No effects of treatment were noted on numbers of litters, live fetuses, early resorptions, or on sex ratio or post-implantation losses. There were no abortions, premature deliveries, complete litter resorptions, or dead fetuses. Late resorptions were observed at 2.0 mg/kg (12 treated vs 0 controls); however, 11 of these occurred in one litter.

b. Altered Growth: There were no treatment-related alterations in fetal growth.

c. Developmental Variations: There were no treatment-related external or visceral variations.

d. Malformations: There were no treatment-related external, visceral, or skeletal malformations.

The developmental LOAEL was not observed. The developmental NOAEL is 2.0 mg/kg/day.

This study is classified **acceptable/guideline (OPPTS 870.3700a)** and satisfies the requirements for a developmental study in the rat.

C. STUDY DEFICIENCIES: No deficiencies were noted.

APPENDIX

Range-finding study for a developmental toxicity study

Since this is a range-finding study, only a summary is provided to confirm the adequacy of the dose selection rationale used in the definitive developmental toxicity study (MRID 45447324).

In a developmental toxicity range-finding study (MRID 45447323), XR-225 (Gamma cyhalothrin; 100.0% a.i.; Lot/batch #CH519MI87ABISE, TSN101800) in corn oil was administered by oral gavage at a dose volume of 4 mL/kg to 8 pregnant female CD rats/group at nominal dose levels of 0, 1, 5, 10, or 15 mg/kg on gestation days (GD) 6-20. Excessive maternal toxicity was observed in the 5, 10, and 15 mg/kg groups; these animals were euthanized prior to study conclusion. The remaining control and 1.0 mg/kg groups were sacrificed on GD 21; their fetuses were removed by cesarean and examined. An additional 15 animals were added to the study to more accurately assess the dose-response relationship of the test compound; 8 were dosed at 2.5 mg/kg, while the remaining 7 served as concurrent controls. These dams were sacrificed on GD 21; their fetuses were removed by cesarean and examined.

Severe neurological and clinical signs of toxicity were observed in dams at ≥5 mg/kg. These dams were euthanized on GD 7-8. No additional data were collected from these animals.

At 1.0 mg/kg, body weight gains were decreased (↓22%; $p \leq 0.05$) on GD 9-12 and generally throughout treatment (GD 6-21).

At 2.5 mg/kg, incoordinated gait was observed in 2/8 dams compared to 0/7 controls, clear perioral stain was noted in 7/8 animals vs 0/7 controls, and clear or red periocular stain was observed in 1/8 animals vs 0/7 controls. Body weight was decreased (↓7%; $p \leq 0.05$) on GD 21. Body weight gains were decreased (↓19-35%; $p \leq 0.05$) on GD 15-21, GD 6-21, and GD 0-21. Terminal body weights were also decreased (↓7%; $p \leq 0.05$).

The maternal LOAEL is 1.0 mg/kg/day, based on decreased body weight gain. The maternal NOAEL was not observed.

No effects of treatment were noted on numbers of litters, resorptions/litter, or fetuses/litter. There were no abortions, premature deliveries, or complete litter resorptions.

The developmental LOAEL was not observed. The developmental NOAEL is 2.5 mg/kg/day.

This range-finding developmental toxicity study is classified as **acceptable/non-guideline** and satisfies the requirements for which it was intended as a dose-selection rationale.

COMPLIANCE: Signed and dated Data Confidentiality, GLP, Flagging, and Quality Assurance statements were provided.

DATA FOR ENTRY INTO ISIS

Developmental Study - rats (870.3700a)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
128867	45447324	developmental	rat	GD 6-20	oral	gavage	0.1-2.0	0, 0.1, 0.5, 2.0	0.5	2.0	Decr. BW, BWG, FC, clinical signs	Maternal
128867	45447324	developmental	rat	GD 6-20	oral	gavage	0.1-2.0	0, 0.1, 0.5, 2.0	2.0	Not observed		Developmental

I

CYHALOTHRIN BACTERIA/MAMMALIAN ACTIVATION GENE MUTATION ASSAY (84-2)

EPA Reviewer: Irving Mauer, Ph.D. Date: 09/24/03
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 10/08/03
Toxicology Branch, HED (7509C)

TXR No.: 0051566

DATA EVALUATION RECORD

STUDY TYPE: Bacterial system, *e.g.*, *Salmonella-Escherichia coli*/mammalian activation gene mutation assay; OPPTS 870.5100 [84-2]; OECD 471, 472

DP BARCODE: D288053 SUBMISSION CODE: S625041

P. C. CODE: 128807 TOX. CHEM. NO.: None.

TEST MATERIAL (PURITY): XR - 225 ("Supercyhalothrin" technical - Lot No. CH519MI87ABISE, 100% a.i.)

SYNONYMS: X670225. Chemically: Cyclopropanecarboxylic acid, 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl-cyano(3-phenoxyphenyl)methyl ester, [1R-[1-alpha(S*), 3-alpha. (Z)]] - ; TSN 101800.

CITATION: Lawlor, T.E. (2000). *Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay, Preincubation Method With A Confirmatory Assay With XR-225, performed by Covance Laboratories, Inc. (Covance), Vienna, (VA): Covance Study No.: 20830-0-422 OECD (Dow Study ID: 99203), dated March 31, 2000. MRID 45447325. Unpublished.

SPONSOR: Dow Chemical Co., Midland (MI); **for** Dow AgroSciences (DAS) LLC, Indianopolis (IN).

EXECUTIVE SUMMARY:

In independently repeat (initial and confirmatory) reverse gene mutation assays in bacteria (MRID 45447325), cultures of four histidine-deficient (*his*$^-$) strains of *Salmonella typhimurium* (TA98,, TA100, TA1535, and TA1537) and the tryptophan-deficient (*try*$^-$) WP2 *uvr*A strain of *Escherichia coli* were exposed by the preincubation modification of the standard Ames procedure to XR-225 (Lot No. CH519MI87ABISE, 100% a.i., in dimethyl sulfoxide, DMSO). Following incubation for 52 ± 4 hours at 37 ± 2°C at six concentrations ranging from 10 to 5000 µg/plate, revertants to histidine and tryptophan prototrophy (*his*$^+$, *try*$^+$) were scored and compared to the incidences in concurrent control (DMSO) cultures. Additional cultures were treated with strain-specific mutagens, to serve as positive controls.

In the range-finding test, cytotoxicity (as evidenced by thinning of background lawn) was observed in non-activated (-S9) TA-100 cultures at ≥ 1000 µg/plate, but not at any dose up to the limit, 5000 µg/plate, in either S9-activated TA100 or WP2 *uvrA*⁻. Slight precipitation of the test material was also found with both tester strains at ≥ 333 µg/plate, progressing to moderate at 3330 and 5000 µg/plate ± S9.

In the mutagenicity assays, however, no increase in test plate revertants over controls were observed in any strain at any dose, in the presence or absence of S9- mix activation. All positive controls responded with marked increases in revertants.

Therefore, XR-225 (supercyhalothrin) is considered non-mutagenic in the standard battery of bacterial strains.

This study is classified as **acceptable/guideline** and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test Material:** XR-225
Description: Off-white odorless solid.
Lot/Batch No.: CH519MI87ABISE
Purity: 100% a.i. (as stated in MRID 45447325, p. 38 and APPENDICES Tables 1 and 2, pp. 41, 42: " measured by: ..... GC ... .HPLC ..... IR/MS [and], Proton and Carbon NMR).
Stability of compound: Stable in solvent (see Section II.A.)
CAS No.: 76703-62-3 (as found in MRID 45447326).
Solvent used: Dimethyl sulfoxide (DMSO).
Other comments: Stored in a sealed container at ambient temperature.

2. **Control Materials:**
Negative: None.
Solvent/Final concentration: DMSO/50 µL.
Positive: **Non-activation:**

Sodium azide	2.0	µg/plate for	TA100, TA1535
2-Nitrofluorene	1.0	µg/plate for	TA98
ICR-191[1]	2.0	µg/plate for	TA1537
Other: 4-Nitroquinoline-N-oxide	0.4	µg/plate for	WP2 *uvr*A

Activation:

2-Aminoanthracene (2-anthramine)	2.5	µg/plate for TA1535, TA100, TA1537; 20 µg/plate for WP2 *uvr*A
Benz(a)pyrene (BaP)	2.5	µg/plate for TA98

[1]ICR 191 = 6-Chloro-9-(3-((2-chloroethyl)amino-)propyl)amino-2-methoxy-, dihydrochloride.

3. **Metabolic Activation**: S9, purchased from Molecular Toxicology, Inc., was derived from hepatic homogenates (post-mitochondrial microsomes) of Aroclor 1254-treated male Sprague-Dawley rats.

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobarbital		non-induced		mouse		lung
	none				hamster		other
	other						other

Describe S9-mix composition:

Component	Amount (= 1.00 mL)
Water	0.70 mL
1 M NaH_2PO_4/Na_2HPO_4, pH 7.4	0.10 mL
0.25 M Glucose-6-phosphate	0.02 mL
0.10 M NADP	0.04 mL
0.825 M KCl/0.2 M $MgCl_2$	0.04 mL
S9 Homogenate	0.10 mL

4. **Test Organisms**: *S. typhimurium* strains (from Dr. B. Ames, UCal, Berkeley).

	TA97	x	TA98	x	TA100		TA102
	TA104	x	TA1535	x	TA1537		TA1538

List any others: *E. coli WP2 uvr*A, from the National Collection of Industrial Bacteria, Torrey Research Station, SCOTLAND (UK).

Properly maintained? Yes.

Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.
Media:

a. **Culturing Broth**:Vogel-Bonner salt solution, supplemented with 2.5% (w/v) Oxoid Nutrient Broth No.2;

b. **Agar Plates**: Bottom agar (25 mL per 15 x 100 mm Petri dish) was Vogel-Bonner minimal medium E, supplemented with 1.5% (w/v) agar and 0.2% glucose; and

c. **Overlay (top): Agar for Selection of Revertants** : Prepared with 0.7% agar (w/v) and 0.5% NaCl (w/v), and supplemented with 10 mL of **either**: 0.5 mM histidine/biotin solution per 100 mL agar for selection of histidine revertants, **or**: 0.5 mM tryptophan solution per 100 mL agar for selection of tryptophan revertants. For an agar overlay; 2.0 mL of the supplemented top agar was used.

5. **Test Compound Concentrations Used:**

Cytotoxicity (Range-Finding) Test (with TA100 and WP2 μvrA ± S9; one plate per dose):
6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1000, 3330, 5000 µg/plate.

Main Assays (all test strains ±S9); 3 plates per dose per strain:
10.0, 33.3, 100, 333, 1000, 5000 µg/plate.

B. TEST PERFORMANCE:

After determination of the dose levels for the main assays (by growth-inhibition effects in TA100 and WP2 *uvr*A, *i.e.*, decreases in reversion per plate and/or thinning of bacterial background "lawn" in the range-finding test), all strains were exposed in triplicate to the test article by a modification of the standard plate incorporation Ames Test (see Section B.1, next), which included prior incubation of the test article, tester strain and S9-mix (for activation tests), or phosphate buffer (for non-activated tests), followed by addition of molten selective agar to this reaction mixture, and overlaying onto minimal agar plates. All plates were then further incubated at 37 ± 2°C for 52 ± 4 hours, at which time revertants on test article plates were counted manually and compared to concurrent solvent values.

The condition of the background lawn was evaluated for cytotoxicity and test article precipitation. The numbers of revertants in positive control plates were counted by an automated colony counter.

1. **Type of *Salmonella* Typhimurium Assay:**

	standard plate test
x	pre-incubation test (20 ± 2 minutes at 37 ± 2°C)
	"Prival" modification
	spot test
	other

2. **Criteria:**

Criteria for assay acceptance and assessment of response were both presented.

II. REPORTED RESULTS:

A. ANALYSIS:

Two analyses of the test article in DMSO revealed averages of ~ 100% for target concentrations ranging from 200 to 100,000 µg/mL.

B. PRELIMINARY CYTOTOXICITY (RANGE-FINDING) ASSAY:

Dose-related precipitation of test substance was evident at ≥ 333 µg/plate, but no cytotoxicity as determined by decreases in revertants up to the limit dosing, 5000 µg/plate (MRID 45447325, p. 30, 31 - ATTACHMENT Tables 1, 2). There was, however, evidence of toxicity as indicated by a thinning of the background lawn of growth at ≥ 1000 µg/plate -S9.

C. MUTAGENICITY ASSAYS:

Precipitation of test article was observed on plates at ≥ 333 µg/plate in both trials, but no increases in revertant counts over concurrent vehicle (DMSO) control values at any dose up to the limit, 5000 µg/plate, in any test strain, either in the presence or absence of S9-mix activation (MRID 45447325, pp. 32 to 35 - ATTACHMENT Tables 3 to 6).

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The EPA reviewers agree with the investigator's conclusions that XR-225 ("superhalothrin" technical) is not mutagenic in a standard battery of *S. typhimurium-E. coli* strains assayed up to precipitation limit dosing.

B. **STUDY DEFICIENCIES**: None.

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CYHALOTHRIN MICRONUCLEUS (84-2)

EPA Reviewer: Irving Mauer, Ph.D. Date: 09/29/03
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 10/08/03
Toxicology Branch, HED (7509C)

TXR No. 0051566

DATA EVALUATION RECORD

STUDY TYPE: *In vivo* mammalian cytogenetics - micronucleus assay in the mouse; OPPTS 870.5395 [84-2]; OECD 475

DP BARCODE: D288053 SUBMISSION CODE: S625041

P. C. CODE: 128807 TOX. CHEM. NO.: None.

TEST MATERIAL (PURITY): XR - 225 (CH519MI87ABISE, TSN 101800, 100% a.i.)

SYNONYMS: X670225; cyhalothrin (ISO suggested common name]. **Chemically**: Cyclopropanecarboxylic acid, 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl-, cyano(3-phenoxyphenyl)methyl ester, [1R-[1.alpha.(S*),3.alpha.(Z]]-

CITATION: Day, S.J., Hammond, T.A. and Beuthin, D.J. (2000). Evaluation of XR-225 in the Mouse Bone Marrow Micronucleus Test, performed at the Toxicology and Environmental Research and Consulting Laboratory, Dow Chemical Company, Midland (MI). Laboratory Project Study ID 991088, dated 19 June 2000. MRID 45447326. Unpublished.

SPONSOR: Dow AgroSciences (DAS) LLS, Indianapolis (IN).

EXECUTIVE SUMMARY:

In an *in vivo* mammalians cytogenetics (micronucleus) assay (MRID 45447326), groups of mice (each, six males and six females) were administered XR-225 (Lot No. CH519MI87ABISE, TSN 101800, 100% a.i., dissolved in 10 mL/kg corn oil) by oral gavage on two consecutive days at doses of 1, 2 and 4 mg/kg/day, and sacrificed 24 hours after the second dose. Equal numbers of mice (6M:6F) were administered two consecutive doses of the corn oil vehicle, or two consecutive doses of 120 mg/kg cyclophosphamide monohydrate, and sacrificed 24 hours after dosing, to serve as negative (vehicle) and positive controls. At sacrifice, femoral bone marrow from each animal was processed cytologically and the frequencies of micronucleated polychromatic erythrocytes (MNPCE) from XR-225-treated mice were compared to vehicle control values.

In prior dose range-finding tests (4 mice/sex) at: (i) twice 10, 50, or 200 mg/kg/day; or (ii) twice 5 mg/kg/day; or (iii) twice 0.5, 2.5, or 5 mg/kg/day), severe signs of toxicity (tonoclonic convulsions, uncoordinated gait, hind limb splay, etc.) were observed at levels above 2.5 mg/kg/day, and deaths at ≥ 5 mg/kg/day.

In the main micronucleus assay, dose-related adverse clinical effects (perineal soiling, and/or increased "reactivity", occasional uncoordinated gait, convulsions) were observed at the mid (2 mg/kg/day) and high (4 mg/kg/day) dosages, but no effect on erythropoiesis (% PCE). **There were no statistically significant increases in the frequencies of MNPCEs in test article-treated mice compared to those in negative control animals**. The positive control animals manifested statistically significant decreases in percentage of PCE, and statistically significant increases in MNPCEs.

This study is classified as **acceptable/guideline** and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vivo* cytogenetic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test Material**: XR-225.
Description: Off-white, odorless solid.
Lot/Batch No.: CH519MI87ABISE, TSN 101800.
Purity: 100% a.i. (by HPLC, GC, 1R/MS, Proton and Carbon NMR - see Section II.1.).
Stability of compound: Stable in vehicle (see Section II.1.).
CAS No.: 76703-62-3.
Solvent used: Corn oil
Other comments: None.

2. **Control Materials**:

Negative/Route of Administration: None.
Vehicle/Final Volume/Route of Administration: Corn oil/10 mL/kg/oral gavage.

Positive/Final Dose(s)/Route of Administration: Cyclophosphamide monohydrate (CP)/120 mg/kg/oral gavage.

3. **Test Compound Administration**:

Volume: 10 mL/kg
Route of Administration: Oral gavage.
Dose levels used:

a. Range-Finding Tests (4M:4F/ twice mg/kg/day):

Phase		
I	II	III
10, 50, 200	5	0.5, 2.5, 5.0

b. Micronucleus Assay (6 M/6F twice mg/kg/day): 1, 2, 4.

4. **Test Animals**:

a. Species: Mice Strain: CD-1 (ICR)BR Age: 8 weeks
Initial Mean Weight: Male: 33.5-34.2 g Female: 25.9-26.2 g
Source: Charles River, Portage (MI)

b. Number of animals used per dose: 6 Males 6 Females [plus 6/sex at the highest dose, for replacement of mice that may die].

c. Properly maintained? Yes.

B. TEST PERFORMANCE:

1. **Treatment and Sampling Times**:

Test compound, vehicle (corn oil), and positive control (CP).

Dosing: ____ once __x__ twice (24 hr apart)

____ (describe): None.

Sampling (after last dose): ____6 hr ____12 hr __x__24 hr

____48 hr ____72 hr (mark all that are appropriate).

2. **Tissues and Cells Examined**:

__x__bone marrow ____other (list):

Number of polychromatic erythrocytes (PCE) examined per animal: 2000.

Ratio of PCE:NCE erythrocytes examined per animal: 200 .

Other (if other cell types examined, describe): None.

3. **Details of Slide Preparation**:

Femoral bone marrow was aspirated into a centrifuge tube containing 0.5 mL fetal bovine serum, centrifuged for 5 minutes, following which the cell pellet was smeared onto standard glass microscope slides, air-dried, and stained with Wright-Giemsa.

4. **Statistical Methods:**

Raw MNPCE data were first transformed (by adding one to each count, then taking the natural log), and sexes analyzed separately using two-way ANOVA (when both sexes were used), or a one-way ANOVA (when only males were used). If dose x sex interaction in the two-way analysis was significant, a one-way ANOVA was performed for each sex. If pairwise comparison of treated vs. control groups was significant (by Dunnett's t-test), a one-sided analysis (upper) for MNPCEs and a two-sided for % PCE were performed. Linear dose-related trend tests were also performed if any pairwise comparisons yielded significant differences. The alpha level for all tests was 0.05.

5. **Criteria:**

Criteria for assay validity as well as response were both presented.

6. **Body Temperature Collection:**

The body temperatures of treated animals were monitored using programmable transponders (Bio-Medic Data Systems, Seaford, DE), immediately prior to dosing, as well as 2 to 6 hours after dosing, and prior to sacrifice. Monitoring of body temperatures provided data to assess transient hypo- and hyperthermia, both of which have been shown to induce micronuclei (Asanami and Shimono, 1997a, b[1,2]; Asanami *et al.*, 1993[3]).

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[1]Asanami, A., and Shimono, K. (1997a) High body temperature induces micronuclei in mouse bone marrow, Mutat. Res., 390, 79-83.

[2]Asanami, A., and Shimono, K. (1997b) Hypothermia induces micronuclei in mouse bone marrow cells, Mutat. Res., 393, 91-98.

[3]Asanami, S., Shimono, K., and Kaneda, S. (1998) Transient hypothermia induces micronuclei in mice, Mutat. Res., 413, 7-14.

II. REPORTED RESULTS:

A. ANALYSIS:

The concentrations of test material in the dosing solutions of corn oil, as well as its homogeneity, were verified by high performance liquid chromatotrophy (HPLC), using UV detection and external quantitation, by the Analytical Laboratory at Dow Chemical Company, and ranged from 98% to 101% of the targeted values (MRID 45447326, p. 49 - ATTACHMENT Table 12).

B. RANGE-FINDING TESTS:

There were no appreciable changes in body weight or temperature of test animals compared to corn oil controls (MRID 45447326, pp. 28 to 33; 45-48, Tables 3, 10, 11). Clinically adverse toxicity (tonoclonic convulsions and repetitive behavior on Day 1 and uncoordinated gait on Day 2) was observed at levels of 2.5 mg/kg/day (MRID 45447326, Tables 4 to 9), and deaths at ≥ 5 mg/kg/day (p. 27, ATTACHMENT Table 2).

C. MICRONUCLEUS ASSAY:

There were no appreciable changes in body weights or temperatures of XR-225-treated animals compared to solvent controls (MRID 45447326, Tables 15, 16, 19, 20). Additionally, no increased incidences of MNPCEs (or significant changes in the ratio of PCE:NCE) in test-treated animals over concurrent corn oil controls were found up to levels causing severe clinical toxicity and/or death (MRID 4544736, pp. 50, 51 - ATTACHMENT Tables 13, 14), nor when compared to historical laboratory controls (p. 64 - ATTACHMENT Table 21). By contrast, animals treated with the clastogen, CP, manifested statistically significant increases in MNPCE frequencies as well as decreases in % PCEs (MRID 45447326, Tables 13 to 16), but no change in body weight or temperature.

Therefore, the investigators concluded that XR-225 (cyhalothrin) did not produce a significant increase in micronucleated polychromatic erythrocytes in mice treated orally up to levels causing clinical toxicity and/or death, and thus is considered negative for clastogenicity.

III REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The EPA reviewers agree with the investigators' conclusions that XR-225 (technical cyhalothrin) was negative for inducing MNPCEs in CD-1 mice when administered orally up to levels producing severe clinical toxicity (including mortality).

B. DEFICIENCIES:

None.

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CYHALOTHRIN BACTERIA/MAMMALIAN ACTIVATION (84-2)

EPA Reviewer: Irving Mauer, Ph.D. Date: 09/29/03
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 10/08/03
Toxicology Branch, HED (7509C)

TXR No.: 0051566

DATA EVALUATION RECORD

STUDY TYPE: Bacterial system, *e.g.*, *Salmonella-Escherichia coli*/mammalian activation gene mutation assay; OPPTS 870.5100 [84-2]; OECD 471, 472.

DP BARCODE: D288067 SUBMISSION CODE: S628592

P. C. CODE: 128807 TOX. CHEM. NO.: None.

TEST MATERIAL (PURITY): GF-231 (a formulation containing 14.7% XR-225 as the active ingredient).

SYNONYMS: TSN 102161

CITATION: Mecchi, M.S. (2001). *Salmonella-Escherichi coli*/Mammalian-Microsome Reverse Mutation Assay - Pre-incubation Method with a Confirmatory Assay with GF-231, performed at Covance Laboratories, Inc. (Covance). Covance Study No.: 21803-0-422 OECD (Dow Study ID: 001186), dated March 29, 2001. MRID 45447327. Unpublished.

SPONSOR: Dow Chemical Company, Midland (MI), for Dow AgroSciences (DAS) LLC, Indianapolis (IN).

EXECUTIVE SUMMARY:

In independent repeat (initial and confirmatory) pre-incubation reverse gene mutation assays in bacteria (MRID 45447327), cultures of four histidine-deficient (*his*$^-$) strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and the trytophan-deficient (try$^-$) WP2 *uvr*A strain of *Escherichia coli* were exposed to GF-231 (a formulation containing 14.7% XR-225, TSN 102161, dissolved in water), at concentrations up to the limit dose, 5000 µg/plate, in the presence and absence of exogenous metabolic activation consisting of hepatic S9-homogenates from Aroclor-1254 treated male rats. In addition to concurrent vehicle (H_2O) controls, other cultures were treated with strain-specific mutagens. Following plate incubation at 37 ± 2° C for 52 ± 4 hours, the frequencies of revertants to prototrophy (*his*$^-$, *try*$^+$) in test cultures were compared to vehicle control values.

In preliminary dose range-finding tests (with TA100 and WP2 *uvr*A), cytotoxicity (reduced background lawn) was evident at ≥ 333 µg/plate in non-activated GF-231- treated TA100 cultures. Slight precipitation of test substance was observed in both species ± S9 at the two highest doses, 3333 and 5000 µg/plate.

In the main mutagenicity assays, cytotoxicity and precipitation at the same dose levels were observed in test article-treated cultures, but at no concentration up to the cytotoxic-precipitating limit dose were statistically increased frequencies of revertants to histidine or tryptophan prototrophy found in either test substance-treated strain, in the presence or absence of metabolic activation. By contrast, all positive controls exhibited marked increases in revertants.

Therefore, the formulation GF-231 (14.7% XR-225 a.i.) is considered non-mutagenic in this battery of bacterial strains.

This study is classified as **acceptable/guideline** and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test Material**: GF-231 (a formulation containing 14.7% XR-225 a.i.).

Description: Opaque, beige, slightly viscous liquid..
Lot/Batch No.: TSN 102161.
Purity: 14.7% a.i. (by HPLC).
Stability of compound: Considered to be stable in vehicle (see Section II.A.).
Vehicle/solvent used: Water (H_2O).
Other comments: Stored at ambient temperature.

2. **Control Materials**:

Negative: Tissue culture medium.
Solvent/final concentration: H_2O/50 uL/plate.

Positive: Non-activation:

Sodium azide 2.0 µg/plate for TA100, TA1535.
2-Nitrofluorene 1.0 µg/plate for TA98.
ICR-191[1] 2.0 µg/plate for TA1537.

Other (list):
4-Nitroquinoline-N-oxide 0.4 µg/plate for WP2 *uvr*A.

Activation:

2-Aminoanthracene (2-anthramine) 2.5 µg/plate for TA98, TA100, TA1535, TA1537; 25.0 µg/plate for WP2 *uvr*A.

3. **Metabolic Activation**: S9, purchased from Molecular Toxicology, Inc., was derived from hepatic homogenates (microsomes) of male Sprague-Dawley rats.

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[1]ICR-191 is: acridine, 6-chloro-9(3((2 chloroethyl)amino)propyl)amino-2-methoxy-dihydrochloride.

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobarbital		non-induced		mouse		lung
	none				hamster		other
	other						other

Describe S9-mix composition (prepared in-house):

Component	Amount (mL)
H_2O	0.70
1 M NaH_2PO_4/NA_2HPO_4, pH 7.4	0.10
0.25 M Glucose-6-phosphate	0.02
0.10 M NADP	0.04
0.825M KCl/0.2 M $MgCl_2$	0.04
S9 homogenate	0.10
Total	**1.00**

4. **Test Organisms**: *Salmonella typhimurium,* from Professor B. Ames (UCal, Berkeley)

	TA97	x	TA98	x	TA100		TA102
	TA104	x	TA1535	x	TA1537		TA1538

List any others: *Escherichia coli*, WP2 *uvr*A, from The National Collection of Industrial Bacteria, Torrey Research Station, Scotland (UK).

Properly maintained? Yes.

Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. **Test Compound Concentration Used:**

a. Range-finding Tests (with TA100, WP2 *uvr*A, ± S9):

6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1000, 3330, 5000 µg/plate (one plate per dose).

b. Main Mutagenic Assays: (all strains, µg/plate: 3 plates/dose):

Bacterial Strain(s)	Initial		Confirmatory		"Extra"	
	- S9	+ S9	- S9	+ S9	- S9	+ S9
TA's	10.0, 33.3, 100, 333, 1000, 3330, 5000	100, 333, 1000, 3330, 5000	10.0, 33.0, 100, 333, 1000, 3330, 5000	100, 333, 1000, 3330, 5000	-	-
WP2 *uvr*A	100, 333, 1000, 3330, 5000	100, 333, 1000, 3330, 5000	10.0, 33.3, 100, 333, 1000, 3330, 5000	100, 333, 1000, 3330, 5000	100, 333, 1000, 3330, 5000	-

B. TEST PERFORMANCE:

All tester bacterial strains were exposed to GF-231 employing the pre-incubation modification of the Ames Assay (Yahagi *et al.*, 1975[2]; Maron and Ames, 1983[3]), which involves:

1. An initial incubation of test article, tester strain and either S9-mix (for activated cultures) or 0.1 M phosphate buffer (for non-activated cultures) for 20 ± 2 minutes at 37 ± 2 ° C;

2. followed by the addition of molten, selective overlay agar

3. thorough mixing;

---

[2]Yahagi, T., M. Degawa, Y. Seino, T. Matsushima, M. Nagao, T. Sugimura, Y. Hashimoto. Mutagenicity of Carcinogenic Azo Dyes and their Derivatives. *Cancer Letters*: 1: 91-96 (1975).

[3]Maron, D.M. and Ames, B. Revised Methods for the *Salmonella* Mutagenicity Test. Mutation Research, 113: 173-215 (1983).

4. overlaying onto a minimal agar plate; and

5. further incubation at 37 ± 2° C for 52 ± 4 hours, at which time, revertant colonies are counted. All concentrations of test article, vehicle and positive controls were plated in triplicate.

Plates were scored for revertants immediately thereafter or later after holdng at 5 ± 3° C. Cytotoxicity was also evaluated both macro- and microscopically (evident by thinning of background lawn and/or reduction in revertants).

C. DATA:

1. Presentation:

For all replicate platings, the mean revertants per plate and standard deviations were calculated, and results presented in tabular form. Recent historical control data were also presented.

2. Criteria: Criteria for assay acceptance and response were both presented.

II. REPORTED RESULTS:

A. ANALYSES:

Several chemical analyses demonstrated stability of GF-231 in the vehicle (water), with (final) 99% to over 100% of target concentrations ranging from 200 to 100,000 µg/mL.

B. RANGE-FINDING TESTS:

Evidence of cytotoxicity (thinning of background lawn and/or reduced revertant counts) was observed at ≥333 µg/plate - S9 in *S. typhimurium* cultures but not in the presence of S9 activation or WP2 *uvr*A cultures, and precipitation of test article at 3330 and 5000, µg/pate in both bacterial strains (MRID 45447327, pp. 23, 24 - ATTACHMENT Tables 1 and 2).

C. MUTAGENICITY ASSAYS:

Thinning of the background lawn was also found in both assays with S. *typhimurium* strains at ≥333 µg/plate -S9 but not in the presence of S9 activation or with *E. coli*, and precipitation at the two highest doses (3330 and 5000 µg/plate) in both test species. However, in no assay at any concentration of GF-

231 were increases in revertant counts (*his⁻ to his⁺; try⁻* to *try⁺)* in test cultures over water controls observed, in either the presence or absence of S9-mix. All positive controls induced marked increases in revertants. Therefore, the investigator concluded that GF-231 was not mutagenic for the standard battery of bacterial strains under the conditions of this study.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The EPA reviewers agree with the investigator that under the exacting conditions of this study (pre-incubation), GF-231 (the formulation containing 14.7% of the active ingredient, XR-225 technical) did not produce any increase in revertants when assayed in *S. typhimurium* or *E. coli* up to cytotoxicity/precipitating doses.

B. STUDY DEFICIENCIES: None.

ATTACHMENT

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gamma-Cyhalothrin Tox review 0051566

Page _____ is not included in this copy.

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R100721

Chemical:	**Cyclopropanecarboxylic acid, 3-(2-chloro**
PC Code:	**128807**
HED File Code	**13000 Tox Reviews**
Memo Date:	***03/18/2004***
File ID:	**TX0050029**
Accession Number:	**412-04-0235**

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